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Health Assessment Document for Acrolein
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Acrolein is an unsaturated aldehyde which is highly toxic by the inhalation and oral routes and is a strong irritant. The reactivity and toxicity of acrolein is seen at the principal sites of exposure, the gastrointestinal and pulmonary tracts. There is no evidence that below aversive levels, acrolein enters the general circulation. Acrolein is metabolized by liver and lung; metabolism is the principal route of elimination. There is inadequate evidence for acrolein's mutagenicity or chemical interaction with mammaliam germ cells. Animal evidence for carcinogenicity is limited. There is no epidemiological data. Acrolein can produce both embryotoxic and fetotoxic effects, and it is teratogenic under certain conditions.

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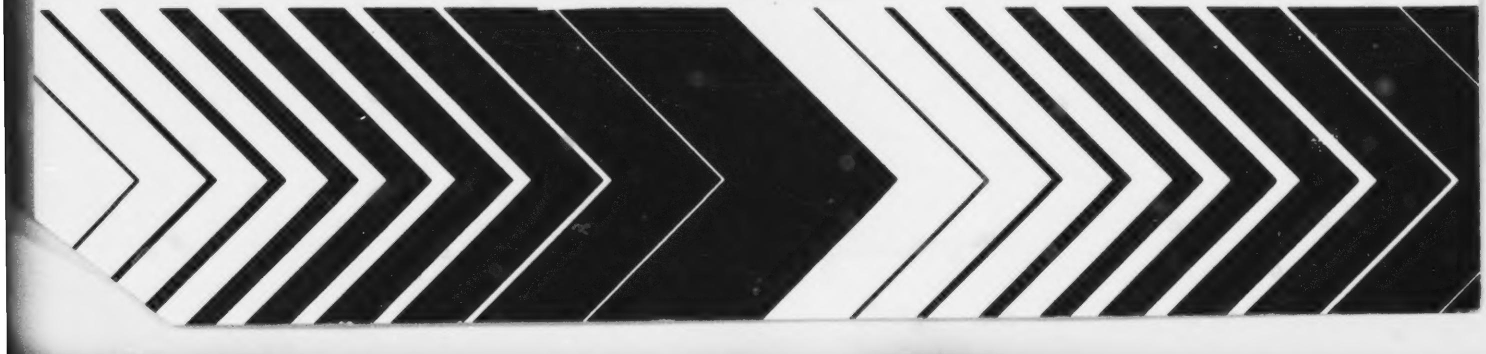

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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
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PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a source document for EPA use. The health assessment was developed for use by the Office of Air Quality Planning and Standards to support decision making regarding possible regulation of acrolein as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels. The relevant literature for this document has been reviewed through July 1, 1986.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

ABSTRACT

This health assessment document on acrolein was undertaken by the Environmental Protection Agency's (EPA) Environmental Criteria and Assessment Office to provide information necessary to determine if regulation of the release of acrolein into the environment may be justified. Acrolein, a chemical intermediate in the synthesis of several organic compounds, has an estimated production volume of 55-70 million pounds per year and a potential for human exposure during manufacture and use.

Acrolein is a volatile, reactive chemical. Its estimated half-life in the atmosphere is less than one day. Studies in animals and the consequences of accidental human exposure indicate adverse effects on the respiratory tract, liver, cardiovascular system, and possibly kidneys. Insufficient data were available to determine dose-response relationships for these effects.

The reactivity and toxicity of acrolein are seen at the principal sites of exposure, the gastrointestinal and pulmonary tracts. There is no evidence that below aversive levels, acrolein enters the general circulation. Acrolein is metabolized by liver and lungs; metabolism is the principal route of elimination. There is inadequate evidence for acrolein's mutagenicity or chemical interaction with mammalian germ cells. Animal evidence for carcinogenicity is limited. No studies of the results of long-term, occupational exposure of humans, or other epidemiological studies were available.

Acrolein can produce both embryotoxic and fetotoxic effects, and is teratogenic under certain conditions. Studies of ecosystem effects show acrolein to be highly toxic to aquatic plants, invertebrates, and fish; calculations suggest that it is not likely to accumulate in the food chain. Recommendations are offered for research in epidemiology, pharmacokinetics, chronic toxicity, and human exposure.

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1. HEALTH EFFECTS SUMMARY AND CONCLUSIONS

1.1 BACKGROUND INFORMATION

1.1.1 Properties

Acrolein is a colorless, volatile liquid at room temperature and has an acrid, pungent odor. It is very soluble in water and soluble in many organic solvents. Acrolein is highly reactive, is unstable in light and air, and can polymerize spontaneously.

1.1.2 Production

Acrolein is currently produced in the United States by the direct oxidation of propylene using one of several catalytic systems. Facilities for the commercial production of acrolein are found in the United States, Federal Republic of Germany, France, and Japan. The sole U.S. manufacturer is Union Carbide; its manufacturing plant has a capacity of 60-100 million pounds per year. Production volume in the United States for 1984 is estimated to be 55-70 million pounds. No estimates were available on the number of persons exposed to acrolein during its manufacture and use.

1.1.3 Use

Acrolein is predominantly used as an intermediate in the synthesis of acrylic acid, allyl alcohol, methionine, 1,2,6-hexanetriol, glutaraldehyde, and, up until 1980, glycerine. Acrolein is used to modify food starch and to control the growth of algae, aquatic weeds, and mollusks. It is also used as a slimicide in the manufacture of paper and as a microbiocide in wastewater injection systems and liquid fuels.

1.1.4 Environmental Release

The potential for the release of acrolein to the environment exists during its manufacture, transport, storage, and use in chemical synthesis as well as its use as a herbicide, however, no data were available on which estimates of the potential levels of release could be made. Acrolein has been identified but not quantified in the process streams of plants manufacturing acrylic acid and oxygenated organic chemicals and in the emissions of processes that remove solvents from coatings using heat. Acrolein is also a component of cigarette smoke, automobile exhaust, wood smoke, emissions from fossil fuel powerplants, and urban smog, and may be released during the cooking or processing of certain foods.

1.1.5 Environmental Transport and Fate

Due to its high vapor pressure and water solubility, acrolein is expected to be highly mobile when released into the environment, although degradative processes are likely to limit its transport. If released into the atmosphere, it is not expected to persist very long, usually one solar day, and would react quickly with hydroxyl radicals and with ozone, to a lesser extent; and also photodissociate. In aquatic systems, acrolein would be removed by chemical and microbial degradation and volatilization. Estimates of acrolein's half-life in water range from 4 to 50 hours. In the terrestrial environment, it is estimated that acrolein would have a low tendency to adsorb to soil and would probably volatilize into air or be leached from the soil by water.

1.1.6 Ecosystems and Aquatic Biota

Several studies have confirmed the effectiveness of acrolein in controlling growth of aquatic plants and bacteria, although effective threshold levels were not identified. The acute toxicity of acrolein to certain aquatic invertebrates and LC_{50} values for several species of freshwater fish (46-240 µg/l) have also been determined. A bioconcentration factor of 344 with a half-life in tissues of more than 7 days was derived from a study in bluegill sunfish. This factor corresponded to a relatively low potential for bioconcentration.

1.2 MAMMALIAN METABOLISM AND KINETICS OF DISPOSITION

Acrolein, is a highly reactive aldehyde which reacts nonenzymatically and enzymatically to 1) form stable adducts with extracellular and intracellular glutathione and other free thiol groups, 2) form adducts with nucleic acids and proteins, 3) cross-links nucleic acids and proteins, and 4) reacts with enzymes and membranes to cause a variety of biochemical consequences such as impairment of DNA replication, inhibition of protein synthesis and mitochondrial respiration, loss of liver and lung microsomal enzyme activities, and other parameters of cellular integrity. This propensity for covalent binding by acrolein provides the basis for its cellular toxicity.

The reactivity and toxicity of acrolein is manifested in the gastrointestinal and pulmonary tracts, the principal sites of exposure. There is no documented evidence (for instance, blood determinations) that for exposure concentrations below aversive levels, acrolein breaches the protective mechanisms at these portals to gain entry to the general circulation. Bronchial and mucosal secretions, and mucosal and endothelial tissues at these locations contain high concentrations of free thiols. Lung, gastrointestinal mucosa and liver also contain effective metabolizing systems with high capacity to biotransform acrolein. Hence the extent and kinetics of absorption of acrolein into the body during oral and inhalation exposure remain to be determined.

Acrolein can be formed in vivo via the metabolism of a number of xenobiotics. For example, allyl alcohol, CH_2CHCH_2OH, causes intensive hepatic periportal necrosis in rats after oxidation by hepatic alcohol dehydrogenase to acrolein. Since protection from allyl alcohol toxicity is afforded by pretreatment with sulfhydryl group donors (cysteine or N-acetylcysteine), it is presumed that when free thiol groups are depleted by acrolein interaction, acrolein combines covalently with other nucleophilic groups of cellular macromolecules and thus leads to cellular damage.

Acrolein has been demonstrated to be metabolized in vivo, particularly by liver and lung parenchymal tissues. Two major pathways have been demonstrated: (1) nonenzymatic and/or glutathione transferase reactions to form stable adducts with glutathione and other thiols leading to mercapturic acid metabolites, and (2) oxidative metabolism to (a) acrylic acid via aldehyde dehydrogenase activity, and (b) to the epoxide glycidaldehyde via microsomal P450 oxidation system. Glycidaldehyde, a reactive metabolite capable of covalent binding, is further metabolized to innocuous metabolites by cellular glutathione epoxide transferase or by epoxide hydrase. The relative importance of these various pathways has not been assessed, nor has the effect of acrolein dosage on metabolic disposition. However, acrolein appears to be extensively, if not completely, metabolized in mammalian systems; acrolein itself has not been found in urine or exhaled air of rodents after parenteral administration of high doses. Hence metabolism appears to be the principal route of elimination from the body. Mercapturic acid derivatives are found in the urine of rodents after administration but account for less than 20 percent of dose. Further information on the distribution of acrolein in vivo, dose-metabolism relationships, profiles of metabolism pathways across species, and relation of covalent binding to toxicity are needed.

1.3 MAMMALIAN TOXICITY

Studies in animals have indicated that acrolein is highly toxic by the inhalation and oral routes and is a strong skin and eye irritant. In subchronic inhalation studies, exposures to levels between 3 and 5 ppm (8 and 12 mg/m^3) caused severe toxic signs and some degree of respiratory damage in all species tested. Studies in mice found that levels below those causing overt toxicity (0.1 ppm) reduced pulmonary compliance and tidal volume. In other studies, inhibition of ciliary transport and decreased resistance to pulmonary infection were reported after short-term exposure to acrolein at 6 ppm. Acrolein has also been found to have effects on the liver (increased enzyme activity), kidneys and cardiovascular system (transient increase in blood pressure) after intravenous injection or inhalation exposure. Subchronic inhalation studies in hamsters, rats, and rabbits (6 hours/day, 5 days/week, 13 weeks) determined a no observed effect level of 0.4 ppm (1 mg/m^3). A chronic inhalation study in hamsters exposed to 4 ppm (7 hours/day, 5 days/week, 52 weeks) indicated increased lung weights and moderate nasal inflammation and epithelial metaplasia; no other levels were tested.

Data on the effects of human exposure to acrolein were limited to short-term irritation studies and to reports of accidental exposures. Acrolein was found to be severely irritating to the mucosa and eyes after a 10-minute exposure at 0.8 ppm (1.8 mg/m^3). The threshold for ocular irritation was 0.2 ppm (0.45 mg/m^3). The consequences of accidental exposure were immediate lung injury and chronic bronchitis and emphysema; two fatalities occurred after inhalation of smoke from burning vegetable oil that contained acrolein vapor. Studies on the effects of long-term exposure were not available for review.

1.4 MUTAGENICITY

The majority of the mutagenicity tests on acrolein have employed bacterial systems and both positive and negative results have been reported. Differences in bacterial strains tested, protocols used and the differences in concentrations tested preclude a reconciliation of the apparently conflicting results. All reports do indicate that acrolein is extremely toxic with significant toxicity noted between 0.1 and 1 μmoles/plate and complete toxicity at less than 5 μmoles/plate in Salmonella.

In eukaryotes, acrolein did not induce gene mutations in methionine requiring strains of yeast, but did induce mitochondrial "petite" mutations in another yeast strain. Acrolein induced sex-linked recessive lethals in Drosophila when larvae were treated but not when adult males were treated in accordance with the currently conventional procedure.

Acrolein did induce sister chromatid exchange in mammalian cells in vitro but only in the absence of exogenous S9 activation. Extreme toxicity precluded detection of chromosome aberrations in these cells. Finally, acrolein was reported negative in a mouse dominant lethal test in which males received a single IP injection of 1.5 or 2.2 mg/kg.

Applying the weight-of-evidence scheme of the proposed Guidelines for Mutagenicity Risk Assessment to the acrolein data, results in the classification of "Inadequate evidence bearing on either mutagenicity or chemical interactions with mammalian germ cells." The basis for this conclusion is the absence of data for mammalian gene mutations and in vivo mammalian cytogenetics data (other than the very limited dominant lethal test), and the lack of data on mammalian germ cell interaction.

1.5 CARCINOGENICITY

Animal evidence for carcinogenicity in skin painting studies is considered to be limited in the evaluation by CAG. There is no epidemiological data. It was not possible, based on the available data, to perform a quantitative risk estimation or to calculate potency factors.

1.6 REPRODUCTIVE AND TERATOGENIC EFFECTS

With respect to reproductive and developmental toxicity acrolein is embryo/fetotoxic. There is no evidence of direct effects on either the male or female reproductive systems. However, the evidence is not convincing that such effects cannot occur since no detailed examination has been done of the effects of acrolein on either the male or female reproductive system. The embryo/fetotoxic effects have been observed in *in vitro* culture and embryo injection studies. The effects include reduced viability and growth retardation. Teratogenic effects can also be produced under specialized conditions. The important consideration appears to be that a sufficient amount of acrolein actually reaches the sensitive sites within the embryo or fetus. Possibly due to binding of acrolein to sulfhydryl and other reactive sites, as well as metabolism of the compound, fetal effects have not been demonstrated *in vivo* in the absence of maternal toxicity.

1.7 REGULATIONS AND STANDARDS

The current standard for acrolein set by the Occupational Safety and Health Administration is 0.1 ppm (0.25 mg/m^3) over an 8-hour workshift. The use of acrolein in food packaging materials and as a starch modifier has been approved by the Food and Drug Administration; the quantity used to modify starch cannot exceed 0.6 percent. Regulations promulgated by the Department of Transportation specify labeling and packaging requirements for acrolein. Under the Clean Water Act, acrolein is a hazardous substance and discharge of more than 1 pound is prohibited. Disposal of acrolein is regulated under the Resource Conservation and Recovery Act, and its use as a pesticide is limited to certified applicators by the Federal Insecticide, Fungicide, and Rodenticide Act.

1.8 CONCLUSIONS

The Environmental Protection Agency is considering the regulation of acrolein as a hazardous air pollutant based on its toxic properties and potential human exposure. The information provided in this document indicates that acrolein's persistence in the atmosphere may be limited to a short period of time and that insufficient data are available to determine current levels of exposure. Studies of acrolein's toxicity in animals suggest that respiratory tract damage occurs after prolonged exposure to low levels of acrolein (4 ppm, 10 mg/m^3). Insufficient data were available to determine the effects of long-term inhalation of acrolein by humans. Further studies are needed to identify effects in humans and to quantitate levels of acrolein in the environment.

1.9 RESEARCH NEEDS

The research needed to support or strengthen the current data base on acrolein is outlined below. Of primary importance are studies that investigate the effects of long-term, low-level exposure in humans.

1. Human Studies - Prospective and/or retrospective cohort epidemiologic studies are needed that consider a range of potential effects on exposed workers and attempt to quantitate exposure.

2. Pharmacokinetics - Studies are needed to verify the reported degree of absorption after inhalation and ingestion by mammals, to identify the distribution of acrolein and its metabolites after absorption.

3. Chronic Toxicity - Chronic inhalation studies, using at least two dose levels, in two species of mammals would be useful to adequately determine acrolein's toxic and carcinogenic effects.

4. Monitoring - Current data on emissions from acrolein's manufacture, transport, and use are needed to permit estimates of general population exposure.

2. INTRODUCTION

This health assessment document has been prepared by the Environmental Criteria and Assessment Office (ECAO) as a basis for its evaluation of acrolein as a hazardous pollutant. It is intended by the Office to be one of several information sources to guide regulatory strategies of the EPA program offices. The preparation of this document involved the participation of the following groups: ECAO, the Carcinogen Assessment Group (CAG), and the Reproductive Effects Assessment Group (REAG), of the U.S. Environmental Protection Agency; and Dynamac Corporation, Rockville, MD. CAG prepared the carcinogenicity section of the document, and REAG prepared the reproductive, teratogenic, and genetic toxicology sections. Dynamac was responsible for the literature search and retrieval and prepared the other sections of the document.

The basis of this document was a literature search performed by the Environmental Control Division of Dynamac Corporation using the health and environmental effects files in the following data base systems: National Library of Medicine (MEDLARS), Lockheed Information System (DIALOG), and System Development Corporation (ORBIT). The literature that was identified in the search was inventoried, and relevant studies were retrieved, evaluated, and summarized. Each chapter was written to include a summary to the significant aspects of acrolein's production, presence in the environment, and/or toxicity.

The major topics included in the document are physical and chemical properties, sampling and analytical methods, production and use, levels and sources in the environment, transport and fate, and biological effects. The discussion of biological effects includes the areas of metabolism and pharmacokinetics as well as mammalian toxicity to organ and tissue systems, carcinogenicity, mutagenicity, teratogenicity, and reproductive effects. Data on the effects of acrolein in humans are also presented.

In the sections on animal toxicity, key studies are presented in a descriptive manner that includes information on the test species, dose or exposure regimen, route of exposure, types of effects seen with each dosage, number of animals in each test and control group, sex and age of the animals, and statistical significance. Information on the purity of the test material is specified when the data were available. Emphasis is placed on observed effect levels and other measures of dose-response relationships.

This document is intended to serve as a basis for decision-making in the various regulatory offices within EPA as well as to inform the general public of the nature and extent of information available for assessment of health hazards resulting from exposure to acrolein.

3. BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Acrolein is an unsaturated aldehyde with the chemical formula $CH_2=CHCHO$. It is a colorless, volatile liquid at room temperature and has an acrid, pungent odor. Acrolein is very soluble in water and soluble in many organic solvents. It is unstable in light and air and can be readily polymerized; hydroquinone is usually added to inhibit polymerization. The conversion factor for acrolein concentration in air is 1 ppm = 2.3 mg/m^3 at 760 mmHg and 25°. Table 3-1 lists the physical and chemical properties of acrolein.

3.1.1 Synonyms

Acrolein has the following synonyms:

- 2-Propenal
- Propenal
- Acraldehyde
- Acrylic aldehyde
- Allyl aldehyde
- Acrylaldehyde
- Prop-2-en-1-al
- 2-Propen-1-one
- Ethylene aldehyde
- Aqualin
- Aqualine
- Acquinite

3.1.2 Identification Numbers

Acrolein has the following identification numbers:

- Chemical Abstracts Service (CAS) No. 107-02-8
- Registry of Toxic Effects of Chemical Substances (RTECS) No. AS 1050000
- Toxicology Data Bank (TDB) No. 0177.

3.1.3 Significance of Physical/Chemical Properties with Respect to Environmental Behavior

Parameters that affect environmental behavior include water solubility, vapor pressure, octanol/water partition coefficient, and degradation rates.

TABLE 3-1. PHYSICAL AND CHEMICAL PROPERTIES OF ACROLEIN

Parameter	Value
Molecular weight	56.06
Specific gravity, 20/20 °C	0.8427
Coefficient of expansion at 20 °C, vol/°C	0.00140
Boiling point, °C	
101.3 kPa (760 mmHg)	53
1.33 kPa (120 mmHg)	-36
Melting point, °C	-87.0
Vapor pressure at 20 °C, kPa (mmHg)	29.3 (220)
Heat of vaporization at 101.3 kPa (1 atm), kJ/kg (Btu/lb)	93 (216)
Critical temperature, °C	233
Critical pressure, MPa (atm)	5.07 (50)
Solubility at 20 °C, wt% (mg/l)	
in water	20.6 (206,000)
water in	6.8
Refractive index n^{20}	1.4013
Viscosity at 20 °C, mPa-s	0.35
Weight per liter at 20 °C, kg (lb/gal)	0.842 (7.02)
Flashpoint, open cup, °C	-18
closed cup, °C	-26
Flammability limits in air, vol%	
upper	31
lower	2.8
Autoignition temperature in air, °C	234
Heat of combustion at 25 °C, kJ/kg (Btu/lb)	5,383 (12,507)
Heat of polymerization (vinyl), kJ/mol (kcal/mol)	71.1-79.5 (17-19)
Heat of condensation (aldol), kJ/mol (kcal/mol)	41.8 (10)

Source: Hess et al. (1978).

Because acrolein is very soluble in water and has a high vapor pressure, its environmental behavior associated with the water and air compartments will probably be of greater importance than its behavior in soil, due to vaporization from the soil into air and leaching from soil into water. The high water solubility and low partition coefficient also suggest that soil sorption may not be an important process (USEPA, 1979). However, since acrolein is very reactive, it is possible that it will bind to organic constituents in the soil and not be available for transport to other environmental compartments. Microbial degradation and reversible hydrolysis appear to be the mechanisms responsible for removal of acrolein from water. The environmental behavior of acrolein is more completely discussed in Section 3.4.

3.1.4 Chemical Reactions in the Environment

Acrolein is a highly reactive chemical because of the carbonyl-double bond conjugation (Hess et al., 1978) and the presence of both a vinyl group and an aldehyde group on a low molecular weight compound (USEPA 1980a). When exposed to light and air, liquid acrolein polymerizes to form disacryl, which is an inactive plastic substance (Merck, 1983).

Acrolein vapor reacts with hydroxyl radical (Edney et al. 1982; Atkinson, 1986) and with ozone (Atkinson et al., 1981). In general, the promary removal process for acrolein in the atmosphere is through reaction with hydroxyl, giving it a lifetime of approximately 14 hours. The compound would be stable to hydroxyl attach at night but would be subject to reaction with ozone.

3.2 ANALYTICAL METHODOLOGY

3.2.1 Chemical Analysis in Air

Prior to the late 1970's, the methods used for sampling acrolein in air involved liquid absorbing solutions. The analytical method of the National Institute for Occupational Safety and Health (NIOSH) is representative of the liquid sorbent techniques (NIOSH, 1978). In this method, air is drawn through two midget impingers with fritted glass inlets containing a mixed absorbing reagent. The reaction of acrolein with 4-hexylresorcinol in the presence of ethanol, trichloroacetic acid, and mercuric chloride results in a blue-colored product with a strong absorption maximum at 605 nm. A spectrophotometer is then used to quantitate the acrolein levels. A concentration of 0.01 ppm acrolein can be detected in a 50-liter air sample using this method. Slight interference may occur with dienes.

In reviewing the NIOSH method, Hemenway et al. (1980) pointed out a potential problem in the sample preparation procedure. The procedure calls for the use of a mixed reagent, and since the trichloroacetic acid, one of the components of this reagent, can react with the other reagents in the solution, it is possible for the mixed reagent to deteriorate over time. According to the authors, actual levels of acrolein may be underestimated by as much as 35 percent because of this problem.

Liquid sorbent procedures are not convenient for personal sampling in an industrial setting or for field sampling. As a result, several solid sorbent sampling methods have been developed that use gas or liquid chromatographic analysis.

A personal air sampling method for acrolein has been developed based on the use of the solid sorbent Amberlite XAD-2 coated with 2,4-dinitrophenylhydrazine (Andersson et al., 1981). Using this method, acrolein in the range of 0.02-0.52 ppm can be analyzed in 5-liter samples with a recovery of 80-100 percent. The analysis of the chemosorbent for acrolein is performed by high-performance liquid chromatography (HPLC) using an ultraviolet (UV) adsorbance detector. Another personal air sampling method using a Porapak N adsorption tube to trap acrolein with subsequent thermal desorption has been described by Campbell and Moore (1979). With this method, acrolein concentrations below 1 ppm can be determined with recovery efficiencies approaching 100 percent. Analysis is performed using a gas chromatograph equipped with a flame-ionization detector (FID).

Hurley and Ketcham (1978) have described a personal air sampling method for acrolein based on the use of hydroquinone-treated carbon as the solid sorbent. Ethylene dichloride is used to desorb acrolein followed by analysis with a gas chromatograph equipped with a FID. Recovery efficiencies of approximately 80 percent are routinely obtained. The method has a sensitivity of 0.02 ppm acrolein for a 5-liter air sample and is useful for measuring acrolein in the 0.05-5 ppm range.

Another analytical method used to measure acrolein concentrations in air involves the use of microwave spectroscopy (Tanimoto and Uehara, 1975). This method reportedly is more selective and has higher resolution than the colorimetric method. Using this method, exhaust samples are collected through a glass tube packed with phosphorus pentoxide and trapped on a color adsorbent in an acetone-dry ice bath. The adsorbent is heated, and the desorbed gas is introduced into the spectrometer. Preconcentration of acrolein is apparently necessary for levels below 10 ppm. Recovery efficiencies were reported to be low.

Activated 13X molecular sieves have been used as the sorbent for acrolein vapor over a wide range, 3-200 µg/g of sieves. Recovery in the 3-8 µg/g range was 97 percent, while recovery in the 60-200 µg/g range was 90 percent following storage at 0 °C for up to 4 weeks. Analysis involved the desorption of the acrolein with distilled water followed by gas chromatographic (GC) analysis using a FID (Gold et al., 1978). A fluorimetric method has also been developed using molecular sieves to collect acrolein vapor and o-aminobiphenyl as the fluorescent reagent. Measurement of the fluorescence intensity with a spectrophotometer showed that the excitation wavelength at 345 nm was highly selective for acrolein. Use of a molecular sieve 3A in combination with molecular sieve 13X allowed the sampling of large volumes of gas. A recovery efficiency of almost 100 percent was reported when a two-stage bubble system was used to collect the desorbed acrolein. The detection limit for this method is 1 ppb (Suzuki and Imai, 1982).

A CO_2 laser photoacoustic technique has been suggested for the determination of ppb levels of acrolein in air (Loper et al., 1982). In this method, the CO_2 laser absorption spectra for acrolein is measured in a very selective manner, and absorptive interferences due to water vapor or other atmospheric compounds are minimized. Based on results of preliminary testing, the technique appears to be highly suited for detecting acrolein in ambient air. Detection limits of approximately 40 ppb were suggested.

An analyzer specific for acrolein has recently been developed to continuously monitor concentrations between 0.01 and 17 ppm in air for periods up to 8 hours (Reddish, 1982). Although this method does not give "real time" information, the time between sampling and analysis is less than 20 minutes. The analyzer consists of six functional units: (1) a gas scrubber/reaction vessel containing an absorbing reagent similar to that used in the NIOSH method; (2) a multichannel peristaltic pump; (3) a reaction coil maintained at 60 °C; (4) a flowthrough cell (black polytetrafluoroethylene) with a 4-cm path length using fiber optics to facilitate measurement of the transmission/absorbance; (5) a colorimeter capable of measuring the colored complex at 605 nm and capable of accepting fiber-optic light transmission; and (6) a chart recorder to record the concentration/absorbance profile of acrolein. Using this technique, 90 percent accuracy has been achieved when compared to results using the HPLC technique.

3.2.2 Chemical Analysis in Water

Various analytical methods are used to determine acrolein concentrations in water (Table 3-2). These include the direct measurement of acrolein by UV spectroscopy, gas-liquid chromatography (GLC), nuclear magnetic resonance (NMR) spectroscopy, colorimetry, differential pulse polarography, titrimetry, and direct fluorescence spectroscopy (Brady et al., 1977; Kissel et al., 1978).

TABLE 3-2. METHODS FOR ACROLEIN MEASUREMENT

Analytical method	Detection limit	Interferences
NMR (aldehydic proton)	100 mg/l	Few
Colorimetry		
2,4-DNPH	80 µg/l	Many
4-Hexylresorcinol	700 µg/l	Many
Fluorimetry		
Direct	20 mg/l	Very few
J-Acid	20 µg/l	Very few
m-Aminophenol derivative	10 µg/l	Very few
Differential pulse polarography	30 µg/l	Few
Gas chromatography		
Flame ionization	500 µg/l	Very few
Mass spectral	50 µg/l	Very few

Source: U.S. Environmental Protection Agency (1980b).

A more recent gas chromatographic/mass spectrometric (GC/MS) method allows the simultaneous analysis of acrolein in a much shorter period of time (Trussel et al., 1981). This technique uses a single fused silica, open tubular capillary column that improves chromatographic resolution. The column, which measures 0.25 mm x 30 m, is coated with SE-54 liquid phase and is directly interfaced to the ion source of the mass spectrometer. The sensitivity of this technique is below the 1 µg/l level. Field applications of this method have not been documented.

3.3 PRODUCTION, USE, AND RELEASES TO THE ENVIRONMENT

3.3.1 Production

Acrolein was initially produced by the vapor-phase condensation of acetaldehyde and formaldehyde. However, since 1959 acrolein has been produced by the direct oxidation of propylene. Many different catalyst systems have been used that allow greater than 90 percent conversion of propylene. The conversion is highly selective to acrolein with 70-84 percent yields. Other products include acrylic acid, acetic acid, acetaldehyde, and carbon oxides (Hess et al., 1978).

Shell Chemical Company phased out production of acrolein on July 1, 1980, due to economic reasons, and Union Carbide Corporation is currently the only producer of acrolein in the United States (SRI, 1980, 1983). Although the production volume is not currently available, the Union Carbide plant in Taft, LA, has a capacity of 60 million pounds per year, which can be expanded to 100 million pounds per year. Production levels in 1984 were estimated to be 55-70 million pounds (SRI, 1980).

3.3.2 Use

Acrolein is predominantly used as an intermediate in the synthesis of several derivatives including acrylic acid, allyl alcohol, methionine, 1,2,6-hexanetriol, and glutaraldehyde (IARC, 1979). Before 1980, Shell Chemical Company used its acrolein production to produce synthetic glycerine; however, since Shell stopped production, acrolein is apparently not used for this synthesis (SRI, 1977, 1980). In 1979, the demand for methionine in the United States was 69 million pounds. Most of this demand (67 percent) was met by acrolein derivatives. Methionine is predominantly used as a protein supplement in animal feed as well as for pharmaceuticals and cosmetics (SRI, 1980).

Other major derivatives include 1,2,6-hexanetriol, which is used as a humectant and in the manufacture of flexible polyurethane foam, and gluteraldehyde, which is used in leather tanning, in photographic chemicals and X-ray supplies, as a sterilizing agent, and as a tissue fixative for transmission electron microscopy (SRI, 1980). Additional chemicals and chemical products produced from acrolein include 2-hydroxyadipaldehyde, quinoline, pentaerythritol, cycloaliphatic epoxy resins, oil-well additives, and water treatment formulae (IARC, 1979).

Acrolein is also used to modify food starch; as an aquatic herbicide, biocide, and slimicide (IARC, 1979); as a microbiocide in wastewater injection systems; to protect liquid fuels from attack by micro-organisms; to control the growth of algae, aquatic weeds, and mollusks in recirculating process water systems; and as a slimicide in paper manufacturing (Brady et al., 1977; Hess et al., 1978).

Acrolein vapor has been used for tissue fixation for electron microscopy. Results from preliminary studies suggest that acrolein vapor fixation preserves more materials in tissue than other methods, such as those that use glutaraldehyde, sodium tetroxide, or osmium tetroxide (Kawai et al., 1983).

3.3.3 Environmental Release

3.3.3.1 Combustion. Acrolein is produced by the incomplete combustion of gasoline, diesel fuel, and other fuels. Levels ranging from 0.05 to 22.5 mg/m^3 have been measured in automobile engine exhaust (Tanimoto and Uehara, 1975; USEPA, 1980), accounting for approximately 3-10 percent of the total aldehydes emitted (Stahl, 1969).

Acrolein has also been identified in wood smoke. Levels detected in pine smoke ranged from 0.62 to 0.67 mg/g of wood burned (USEPA, 1980). Einhorn (1975) reported levels as high as 50 ppm in smoke from the combustion of wood. Acrolein was also identified (but not quantified) in the air of commercial smokehouses (Love and Bratzler, 1966).

Acrolein is suspected to be a gaseous emission from fossil fuel powerplants, as one of the group of chemicals classified as formaldehyde and related compounds (Natusch, 1978). For this aldehyde group, average emission levels of 0.002, 0.1, and 0.2 lb/1,000 lb of fuel have been reported for coal, oil, and natural gas plants, respectively. Emissions of aldehydes from both oil- and gas-powered plants have been reported to be 0.5 lb/ton of fuel. The actual levels of acrolein contained in these emissions has not been determined.

Acrolein has been identified as both a combustion product and a pyrolysis product of polyethylene-based materials (Potts et al., 1978). Acrolein concentrations resulting from combustion of polyethylene foams ranged from 2 to 23 ppm, while levels measured (by GC/MS analysis) during pyrolysis were between 76 and 180 ppm. Acrolein has also been detected during the incineration of plastic beverage containers (Wharton, 1978).

3.3.3.2 Cigarette Smoke. Several studies have confirmed the presence of acrolein in cigarette smoke. Horton and Guerin (1974) reported that a commercial, 85-mm filtered cigarette delivered 102 μg of acrolein (153 μg/g tobacco) directly to the smoker. A similar, nonfiltered cigarette delivered 111 μg of acrolein (135 μg/g tobacco); an experimental 85-mm marijuana cigarette contained 145 μg of acrolein (199 μg/g marijuana); and a commercial 85-mm cigar contained 70 μg of acrolein (107 μg/g tobacco). An experimental 85-mm cigarette with a charcoal filter delivered the least amount of acrolein, 62 μg/cigarette (97 μg/g tobacco).

Acrolein is also present in the sidestream smoke of cigarettes, leading to possible exposure for nonsmokers. Ayer and Yeager (1982) reported acrolein levels of 0.9-1.3 ppm in the sidestream smoke of three commercial brands of cigarettes that were three orders of magnitude above occupational limits. In experiments to determine the gas-phase components of sidestream smoke, Jermini et al. (1976) reported that the acrolein concentration in an unventilated 30 m^3 room after the simultaneous smoking of 30 cigarettes was 0.37 ppm.

3.3.3.3 Food Processing. Acrolein is produced as a result of the heating of organic substrates. It has been detected (but not quantified) during the cooking or processing of foods (Boyd et al., 1965; Grey and Shrimpton, 1967; Hrdlicka and Kuca, 1965; Izard and Libermann, 1978; Kishi et al., 1975) and in the fermentation of alcoholic beverages (Rosenthaler and Vegezzi, 1955).

3.3.3.4 Production Processes. No information was found on the release of acrolein during its production or use as a chemical intermediate. Acrolein has been identified in the process streams in acrylic acid plants (Serth et al., 1978), in industries manufacturing oxygenated organic compounds (e.g., aldehydes, alcohols), and in processes that remove solvents from coatings by the use of drying or heating ovens (Stahl, 1969). Acrolein is also evolved during the processing of plastics (temperature range 140-340 °C); however, release rates have not been reported (Reddish, 1982).

3.3.4 Environmental Occurrence

A very limited amount of data is available regarding the environmental levels of acrolein. Levels between less than 1 and 20 mg/m^3 (.44 and 8.7 ppm) are considered representative of concentrations present in urban air (Carson et al., 1981; Natusch, 1978).

Acrolein is a component of urban smog and has been measured in the air of Los Angeles, CA. The average concentration for 10 days during the period September-November 1960 was 0.005 ppm (.011 mg/m^3), and for 7 days during the same period in 1961 it was 0.008 ppm (.018 mg/m^3) (Altschuller and McPherson, 1963). An average concentration of 0.004 ppm (.009 mg/m^3) was reported for 10 days between July and November 1960 (Renzetti and Bryan, 1961).

Propylene is oxidized to acrolein in air. A mathematical model developed by Graedel et al. (1976) has computed the peak concentration for acrolein in urban air based on several variables including estimated rate constants for the reactions leading to the formation of acrolein from the oxidation of propylene (with allylic radicals being the apparent precursors). Based on this model, the peak concentration for acrolein in urban air (Los Angeles) is estimated to be 1.3×10^{-2} ppm.

Acrolein can also be formed in the atmosphere by the photo-oxidation of diolefins or other hydrocarbon nitrogen oxide mixtures (Stahl, 1969).

Acrolein has not been shown to be a contaminant of drinking water or water supplies (USEPA, 1980b).

Although acrolein has been detected in many foods (see section 3.3.4.3), the concentrations have not been quantified.

3.4 ENVIRONMENTAL TRANSPORT AND FATE

Acrolein is released into the atmospheric, aquatic, and terrestrial environments. Acrolein is released into the atmosphere from the manufacture, transport, use, or combustion of organic substrates; into natural waters from manufacturing effluents and direct herbicidal use; and onto land as a result of accidental spills, indirect herbicidal use, and land disposal. Once released, the environmental fate of acrolein is determined by a combination of dispersive, degradative, and accumulative processes. The relationships of these processes to acrolein's environmental transport and fate are discussed in the following sections.

3.4.1 Transport

Acrolein is expected to be highly labile when released into the environment. Because of its high reactivity, its transport in the environment is limited. Due to its high vapor pressure, if spilled, it is expected to rapidly evaporate at ambient temperatures. Once in the atmosphere, it will react with OH radicals, ozone, and photodissociate.

Acrolein is very soluble in water (206,000 mg/l). Due to rapid degradation, however, only between 2 and 29 percent of the amount of acrolein released into a river is estimated to be transported downstream to a distance of 50-250 miles over a 5-day travel period (Falco et al., 1980). In laboratory experiments, Bowmer et al. (1974) found that differences in the chemical properties of water bodies could affect the loss of acrolein by reaction or degradation, whereas greater turbulence is expected to increase loss by volatilization.

The extent to which acrolein is transported through the terrestrial environment is inversely related to the degree to which it is adsorbed to soil. Soil adsorption can be described by the soil/water partition coefficient as a function of organic carbon content (K_{oc}). This value may be estimated for acrolein by the regression equation of Kenaga and Goring (1980). Using this equation [$\log K_{oc} = 3.64 - 0.55 (\log S)$, where S = water solubility in mg/l], a K_{oc} value of 5.2 is estimated, indicating a low soil adsorption potential for acrolein. Thus, acrolein is expected to volatilize from soil and/or leach from soil where it may be transported to groundwater.

3.4.2 Fate

3.4.2.1 Atmospheric Fate. Few data were found on the atmospheric fate of acrolein. The available information suggests that acrolein released into the atmosphere would persist for a few days only. Aldehydes in the atmosphere, in general, can be expected to photodissociate into the "R" group and a free aldehyde group. This "Norrish Type 1" fragmentation typically occurs at 313 nm irradiation (Calvert and Pitts, 1966).

The primary photochemical process, however, would compete with possible photophysical deactivation processes to affect the excited-state molecules. Moreover, the radicals produced may undergo secondary reactions to yield thermally stable compounds (Calvert and Pitts, 1966).

Cupitt (1980) described two chemical removal processes in air that affect organic compounds containing double bonds such as acrolein. The first is reaction with hydroxyl radicals that may be added across the double bond of acrolein to form oxygenated compounds such as aldehydes, ketones, and dicarbonyls. The second chemical removal process is reaction with ozone. This process (ozonolysis) results in the formation of a carbonyl compound (aldehyde or ketone) and a percarbonyl biradical that may undergo further rearrangement to form a variety of products including organic acids or radicals and carbon dioxide.

In a laboratory study to determine atmospheric hydroxyl reactions, Edney et al. (1982) reported that both additions and abstractions by OH radicals were mechanisms involved in the removal of acrolein from air. Reaction products identified included peroxy nitrates, formaldehyde, and glycoaldehyde. An atmospheric half-life for acrolein, based on the hydroxyl rate constant was estimated to be 5.6 hours.

The atmospheric residence time (T) can be estimated for acrolein according to the method of Lyman et al. (1982). The method estimates residence time as a function of reaction rates with hydroxyl radicals and ozone but does not consider reactions with other substances or photodissociation; therefore, T must be considered a maximum rate.

Acrolein is removed from the atmosphere thru OH and O_3 reactions. The ambient levels of OH are of the order of 10^6 molecules/cc. Since the rate constant is 20×10^{-12} cc mol^{-1} sec^{-1}, the lifetime is approximately 14 hrs. Acrolein will also react with ozone to the same extent but since the rate constant is of the order of 10^{-19} cc mol^{-1} sec^{-1} with ambient levels of O_3 at 40-50 ppb, this reaction will not be very important for the removal of significant levels of acrolein. Acrolein will also photodissociate with an atmospheric lifetime of 5.3 days. Thus, the most important removal process of acrolein is thru reactions with OH radicals (U.S. EPA, 1986).

3.4.2.2 Aquatic Fate. Acrolein may be removed from natural waters through a combination of physical, chemical, and biological degradative processes and by volatilization, sorption, and dilution. Information on the relative importance of the individual processes is limited; therefore, this discussion is supplemented with extrapolations from known properties of acrolein.

Aqueous photolysis is a possible degradative mechanism for acrolein in water. As discussed in the previous section on atmospheric fate, the primary photochemical reaction would be a "Norrish Type 1" fragmentation, which typically occurs at 313 nm irradiation. For acrolein in the environmental radiation range of 290-700 nm, the maximum absorption will occur at 315 nm with a molar absorptivity (E) equal to 26 liters/mol-cm (Lyman et al., 1982). These data, however, are not sufficient to predict aqueous photolysis rates, in part because they may be affected by suspended sediments, surfactants, and sensitizers.

The United States Bureau of Reclamation reported that acrolein dissipation from flowing water is a first-order process (Bowmer and Sainty, 1977). The equation is:

$$K = (U/\Delta X) \ln (C_a/C_b)$$

where K is the first-order rate constant of decay; C_a and C_b are the concentrations observed in the plateau (i.e., stable concentration) region as the treated water passes stations at distances X_a and X_b downstream from the injection point; and U is the mean velocity. The first-order rate contants were determined in eight irrigation canals in Australia and the United States (Bowmer and Sainty, 1977). K values ranged from 0.104 to 0.211 per hour and had a mean (± standard deviation) value of 0.16 (± 0.04) per hour. This corresponds to a half-life of 4.3 hours. It was hypothesized that acrolein dissipated by volatilization, degradation, and adsorption.

The primary acrolein reaction products have been identified in laboratory studies under acidic and alkaline conditions (Bowmer and Higgins, 1976). The primary reaction of acrolein under acidic conditions is a reversible hydrolysis to beta-hydroxypropionaldehyde, whereas under alkaline conditions a polycondensation reaction occurs to form a pentamer. In their study using environmental pH conditions (pH 5-9), reaction products were not identified. Bowmer and Higgins predicted that acrolein's half-life in the Australian irrigation canals would be pH dependent (38 hours at pH 8.6 and 50 hours at pH 6.6).

The third degradative process that may be important to the aquatic fate of acrolein is biodegradation. Acrolein has been reported to be relatively nonbiodegradable. In a determination of the oxygen demand (i.e., degradability) of chemicals of interest to Shell Research Company (Amsterdam), Bridie et al. (1979) reported that the theoretical, 5-day biochemical (BOD), and chemical oxygen (COD) demands of acrolein to be 2.0, 0.0, and 1.76 g/g, respectively. According to Lyman et al. (1982), biodegradability may be estimated from the ratio of 5-day BOD to COD. Acrolein with a value of 0.01 is thus rated as "relatively undegradable" using the data of Bridie et al. and the degradability index of Lyman et al. solubility and low partition coefficient also suggest that soil sorption may not be an important process (USEPA, 1979). However, since acrolein is very reactive, it is possible that it will bind to organic constituents in the soil and not be available for transport to other environmental compartments. Microbial degradation and reversible hydrolysis appear to be the mechanisms responsible for removal of acrolein from water. The environmental behavior of acrolein is more completely discussed in Section 3.4.

The "microbial degradation rate" of acrolein in a hypothetical river was calculated using the EXAMS model by Falco et al. (1980). The rate of 0.08/day suggests that acrolein is probably somewhat more biodegradable than indicated above.

Bowmer and Higgins (1976) studied the persistence and fate of acrolein in several Australian irrigation canals. An unidentified, nonvolatile reaction product of acrolein was observed when acrolein concentrations fell below 2-3 ppm. It was transient and dissipated rapidly following a lag period reportedly through "microbiological processes." The authors suggested that these processes might involve microbial oxidation of the aldehyde to carboxylic acid.

Actual measurements of acrolein volatilization from water were not found in the literature. Estimates of the liquid and gas exchange coefficients indicate the importance of volatilization to overall acrolein dissipation. According to Lyman et al. (1982), when the Henry's Law Constant (H) ranges from 10^{-5} to 10^{-3} atm-m^3/mol, as with acrolein, both the liquid-phase and gas-phase resistances are important and volatilization is moderate. If it is assumed that there are no resistances other than those of the gas and liquid phases, the half-life of acrolein in water at depth Z (e.g., 100 cm) is 5.5 hours. However, volatilization would be limited by chemical reactions or degradation of acrolein in water, whereas turbulence is expected to increase loss by volatilization (Bowmer et al., 1974).

Acrolein present in natural waters is not expected to adsorb to bottom sediments or to bioconcentrate in significant quantities. The estimated low sediment (or soil) adsorption potential has been discussed in section 3.4.1, Transport. The bioconcentration of acrolein in the bluegill sunfish was studied and, as predicted from acrolein's chemical properties, was not found to be a very significant process (Veith et al., 1980). Bioconcentration and other accumulation processes are discussed further in chapter 9, Ecosystem Considerations.

3.4.2.3 Terrestrial Fate. As discussed in the transport section, acrolein has a low soil adsorption potential. Acrolein is expected to volatilize or leach from the soil where it will be transported either to the atmosphere or to groundwater.

3.5 ECOSYSTEM CONSIDERATIONS

3.5.1 Introduction

Because of its use as an aquatic herbicide and as an organic intermediate, acrolein may be potentially harmful to the aquatic and terrestrial ecosystems. Since the 1950's the effects of acrolein on various target and nontarget organisms have been considered and reported in the scientific literature. The effects on residents of the aquatic ecosystem, fish and some aquatic plants in particular, are best known (Section 3.6). Very little is known about acrolein's potential effects on terrestrial ecosystems (Section 3.7). Acrolein has a relatively limited potential for bioconcentration and bioaccumulation (Section 3.8).

3.6 AQUATIC ECOSYSTEMS

The effects of acrolein on aquatic life are summarized in the sections below. The available literature was limited for all groups except fish. The data indicate that acrolein has acute toxic effects on most fish and aquatic invertebrates tested at a concentration of 1 mg/l.

3.6.1 Aquatic Plants, Bacteria, and Algae

The few available studies were designed to show the effectiveness of acrolein for controlling nuisance growths of aquatic plants, bacteria, and algae. Consequently, there was little concern with identifying either threshold response levels for nontarget species or overall ecosystem effects. In general, however, the tested herbicidal or slimicidal levels exceed the safe levels for fish (see Section 3.6.3).

The growth of two aquatic plants, floating pondweed (*Potamogeton tricarinatus*) and ribbonweed (*Vallisneria spiralis*), in several Australian irrigation canals was controlled by acrolein treatments (Bowmer and Sainty, 1977). Laboratory studies confirmed field observations that pondweed is 7-10 times more tolerant of acrolein than ribbonweed. Mature plants of each species growing in buckets of mud were exposed to measured levels of acrolein in 500-liter containers for at least 1 hour followed by "washing and 7 days' growth in clean water." One-hour treatments with 26 mg/l acrolein for pondweed and 3.7 mg/l acrolein for ribbonweed resulted in 80 percent reduction in plant growth, compared to controls, 1 week from treatment.

Van Overbeek et al. (1959) reported that acrolein, as the active ingredient in an unspecified product, controlled submersed aquatic weeds in a 20-mile irrigation canal in Kern County, CA. Application of 1.15 gallons of acrolein per cubic foot of waterflow per second for 30-45 minutes controlled the growth of pondweed (*P. crispus*) and other unspecified weeds for several weeks, and was sufficient to raise water flow by about 75 percent. Van Overbeek and coworkers also reported that in laboratory experiments, leaf cells of the water plant *Elodea densa* were destroyed by acrolein application at 0.5 ppm after 24 hours or at 5 ppm after 2 hours. The authors hypothesized that the mode of action was mediated by effects on enzyme systems with functional sulfhydryl group, rather than cell membrane, destruction. This conclusion was based on the observation that *Elodea* cell contents were destroyed after being dipped in 1,000 ppm acrolein, while the cells maintained turgor pressure for several hours.

The effects of acrolein on aquatic bacteria were reported by Starzecka (1975), who tested the effectiveness of a powerplant cooling water biocide. Several bacterial groups (including Pseudomonas and Achromobacter-Alcaligenes) were isolated from the "unpolluted" Trzebunka River in Poland and tested using high levels (greater than 62 mg/l) of an unspecified formulation of acrolein. Bacterial cultures grown for several days in peptone (10 g/l) and glucose (1 g/l) were slightly inhibited by acrolein at 62 mg/l and severely inhibited at 125-250 mg/l. Levels such as these far exceed the concentrations necessary to control submersed plants (Bowmer and Sainty, 1975; Van Overbeek et al., 1959).

Only one study was found indicating that acrolein could reduce the growth of a green filamentous alga, Cladophora sp. (Jordan et al., 1962). Visual estimates of Cladophora growth were made during 4 months in three replicate ponds (675 ft^2; 7,500 gallons) treated with technical-grade acrolein (at 3 mg/l, nominal). The estimates of percent pond surface coverage by algae made by two to seven independent observers were evaluated for statistical significance by analysis of variance and multiple range tests. In only one of the 4 months was the acrolein treatment found to be significantly different from controls; however, the study did not provide sufficient data to determine a threshold of algal toxicity.

3.6.2 Aquatic Invertebrates

Although acute toxicity data were found for six aquatic invertebrates, only the waterflea (Daphnia magna) was tested using a well-defined protocol with measured levels of acrolein. In addition, data from a chronic study with D. magna showed acrolein to be highly toxic (Table 3-3).

Acute bioassay procedures, recommended by the American Public Health Association, and chronic testing procedures, developed by Macek et al. and personnel of EPA's National Water Quality Laboratory, were used to test acrolein on D. magna (Macek et al., 1976). Proportional dilutions delivered acrolein (99 percent active ingredient) to four replicate jars each containing 2 liters of one of five test concentrations. For both the acute and chronic studies, 20 test vessels each were used, having 5 or 10 waterfleas in each vessel, respectively.

In both the acute and chronic studies, young animals (less than 24 hours old) were used. In the acute studies, survival was measured after 48 hours. In the chronic studies, survival and production of young were recorded at 1, 2, and

TABLE 3-3. EFFECTS OF ACROLEIN ON AQUATIC INVERTEBRATES

Species	Toxicity test[a]	LC_{50} in μg/l (time in hr)[b]	Temp., °C	pH	Hard-ness	Reference
Waterflea (*Daphnia magna*)	Static, laboratory	230 (24) 83 (48)	22 ± 1	7.0-8.0	72-173	LeBlanc (1980)
Waterflea (*D. magna*)	Static, laboratory; measured acrolein levels	57 (48)	20 ± 1	7.0-7.3	35	Macek et al. (1976)
Waterflea (*D. magna*)	Three-generation chronic test; measured acrolein levels	64-day MATC 16.9 33.6	20 ± 1	7.0-7.3	35	Macek et al. (1976)
Oyster (*Crassostrea virginica*)	Flowing seawater, laboratory	50[c] (96)	-[d]	-	-	Butler (1965)
Brown shrimp (*Penaeus aztecus*)	Flowing seawater	100 (48)	-	-	-	Butler (1965)
Snail (*Physa* spp.)	River study	25,000 (4)	-	-	-	Unrau et al. (1965)
Snail (*Biomphalaria alexandrina*)	River study	25,000 (4)	-	-	-	Unrau et al. (1965)
Snail (*Bulinus truncatus*)	River study	25,000 (4)	-	-	-	Unrau et al. (1965)

[a] Acute, freshwater studies with nominal acrolein levels (unless otherwise noted).
[b] Unless otherwise noted.

[c] EC_{50} shell growth.

[d] Data not available.

3 weeks. The young at weeks 1 and 2 were discarded. At week 3, 10 young were selected at random to begin a second-generation test. The same procedures were used for second- and third-generation tests. The three generations were studied for 21-22 days each. Treatment effects were measured by Duncan's multiple range test.

In selected vessels, acrolein was analyzed by adding 2,4-dinitrophenylhydrazine forming a hydrazone complex, which was extracted with benzene and determined spectrophotometrically (365 nm). The minimum detectable level was 3 ng/l. Actual acrolein measurements of 3.2-42.7 µg/l (n=8) agreed fairly well with the five nominal concentrations (4-60 µg/l). Water quality parameters (the mean values ± SD) were also measured: alkalinity (33 ± 2.1, n=5); total hardness (35 ± 2.1, n=5); pH (7.1 ± 0.1, n=5); acidity (4.4 ± 0.8, n=5); temperature (20 ± 1 °C); and dissolved oxygen (7.5 ± 0.6, n=29).

The acute 48-hour bioassay yielded an LC_{50} (95 percent confidence interval) of 57 (20-99) µg/l. Based on the three-generation 64-day test, the maximum acceptable toxicant concentration (MATC) ranged from 16.9 to 33.6 µg/l. The sensitivity of young Daphnia to acrolein was greatest in the second generation, when survival was significantly reduced at exposures of 16.9 µg/l. In the first and third generations, however, Daphnia survival was reduced at concentrations greater than or equal to 33.6 µg/l.

3.6.3 Fish

Acute and chronic studies with several species of fish indicate that exposures to acrolein for several hours at concentrations below 250 µg/l are highly toxic (Table 3-4). At these low levels, acrolein was harmful to all tested fish species: estuarine and freshwater, coldwater and warmwater, juvenile and adult, and traditionally tolerant and intolerant species. Moreover, all species showed similarly low acrolein tolerance levels regardless of experimental conditions (e.g., static vs. flowthrough, measured vs. unmeasured acrolein levels). The 24- to 96-hour LC_{50} values for 11 freshwater species ranged from 46 to 183 µg/l; the single 24-hour LC_{50} value for estuarine killifish was slightly higher, 240 µg/l.

Measured concentrations of acrolein were reported in only two studies. One of these studies (Macek et al., 1976) is discussed below along with a comparatively detailed study (Lorz et al., 1979) in which only nominal concentrations of acrolein were found.

TABLE 3-4. EFFECTS OF ACROLEIN ON FISH

Species	Toxicity test[a]	LC_{50} in µg/l (time in hr)[b]	Temp., °C	pH	Hardness	Reference
Bass, largemouth (Micropterus salmoides)	Static	183 (24) 163 (48) 160 (96)	21-22	7.2-7.3	40-41	Louder and McCoy (1962)
Bowfin - fry (Armia calva)	Static	62 (24)	21-22	7.2-7.3	40-41	Louder and McCoy (1962)
Bluegill (Lepomis macrochirus)	Static; acrolein levels measured	100 (24) 90 (96)	20-24	6.7-7.4	28-44	Buccafusco et al. (1981)
Bluegill (L. macrochirus)	Static;	140 (24) 125 (48) 100 (96)	21-22	7.2-7.3	40-41	Louder and McCoy (1962)
Bluegill - fingerlings (L. macrochirus)	Flowing	79 (24) (TLM)	15	-[c]	-	Burdick et al. (1964)
Fathead minnow (Pimephales promelas)	Flowing; acrolein levels measured	84 (144)	25 ± 1	6.6-6.8	32 ± 4.7	Macek et al. (1976)
Fathead minnow (P. promelas)	Flowing; three-generation chronic test; acrolein levels measured	11.4-41.7 (MATC)	25 ± 1	6.6-6.	32 ± 4.7	Macek et al. (1976)
Fathead minnow (P. promelas)	Static	150 (24) 115 (48)	21-22	7.2-7.3	40-41	Louder and McCoy (1962)
Goldfish (Carassius auratus)	Static; acrolein levels measured	80 (24)	20	-	-	Bridie et al. (1979)
Harlequin (Rasbora heteromorpha)	Flowing	140 (24) 60 (48)	20	-	20	Alabaster (1969)

TABLE 3-4. (continued)

Species	Toxicity test[a]	LC_{50} in µg/l (time in hr)[b]	Temp., °C	pH	Hardness	Reference
Killifish, longnose - juveniles (*Fundulus similis*)	Flowing seawater	240 (24)	-	-	-	Butler (1965)
Mosquitofish (*Gambusia affinis*)	Static	149 (24) 61 (48)	21-22	7.2-7.3	40-41	Louder and McCoy (1962)
Salmon, chinook (*Oncorhyncus tschawytscha*)	Static	80 (24)	20	7.4-7.7	-	Bond et al. (1960)
Salmon, coho (*O. kisutch*)	Static	68 (36)	10	7.4-7.6	100	Lorz et al. (1979)
Trout, brown - fingerlings (*Salmo trutta*)	Flowing	46 (24) TLM	15	-	-	Burdick et al. (1964)
Trout, rainbow - fry (*S. gairdneri*)	Flowing	100 (avoidance after 1 hr)	11 ± 2	8.0	89.5	Folmar (1976)
Trout, rainbow (*S. gairdneri*)	Static	65 (24)	20	7.4-7.7	-	Bond et al. (1960)

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[a] Laboratory studies using freshwater species (except killifish) with nominal acrolein levels (unless otherwise noted.

[b] Unless otherwise noted.

[c] Data not available.

The response of the fathead minnow (Pimephales promelas) to acrolein was studied using acute and chronic bioassay procedures (Macek et al., 1976). Mount-Brungs proportional dilutors were used to dose duplicate glass aquaria (0.08 m^3) with flowthrough rates equaling seven aquarium volumes daily. Diluent well water was analyzed for 28 ions or compounds.

Acrolein (99 percent active ingredient) in 100 percent ethanol was added to each aquarium to achieve the appropriate test concentration. Acrolein was analyzed by adding 2,4-dinitrophenylhydrazine to form a hydrazone complex, which was extracted with benzene and determined spectrophotometrically (365 nm). The minimum detectable level was 3 ng/l. Mean acrolein concentrations in the chronic studies ranged from 4.6 ± 2.7 (n=15) µg/l to 41.7 ± 35.8 (n=28) µg/l. Total hardness, alkalinity, pH, acidity, temperature, and dissolved oxygen were measured by APHA (1971) methods.

Fathead minnows exposed to measured acrolein concentrations (0-41.7 µg/l) for 3-245 days showed no acrolein-related changes in survival, length, or weight. Eighty fish were tested at each concentration. There were also no effects on spawning, number of eggs per female, and percent hatchability among treated and control fish. However, two larval groups of second-generation fatheads exposed to acrolein at 41.7 µg/l for 60 days showed only 2 percent survival, which was significantly lower ($p < 0.05$) than the survival rate for controls and test concentrations up to 11.4 µg/l (Macek et al., 1976).

Static 96-hour LC_{50} values were reported for yearling coho salmon exposed to 1 of 12 water-soluble herbicides including acrolein (Lorz et al., 1979). The estimated 96-hour LC_{50} value for acrolein was 68 µg/l (Table 3-4). Acrolein was the most toxic, being more than two orders of magnitude more toxic than 10 of the herbicides including picloram, atrazine, diquat, and paraquat. Lorz and coworkers concluded that acrolein could kill all salmonid life stages if normally treated irrigation waters were released into streams before herbicide inactivation.

Static tests were performed in fiberglass tanks (120 liters, aerated, 85 percent replacement daily) using at least two replicates of seven acrolein concentrations with 10 fish per tank. The acrolein used was described only as a 1-liter sample provided by Shell Chemical Company. Acrolein concentrations were not measured. The fish used were 12- to 17-month-old coho salmon obtained from Oregon's Fall Creek Salmon Hatchery.

Virtually all (119 of 120) coho yearlings exposed to acrolein at 0-50 µg/l for 144 hours survived, whereas all 40 cohos exposed at 75-100 µg/l for 144 hours died. There was no increase in mortality in the surviving cohos when transferred to seawater for 280 hours.

Histologic examination of the gills, kidneys, and liver from the three fish selected from each group (0, 50, or 100 µg/l) indicated increased incidences of tissue lesions that were dose-dependent (Macek et al., 1976).

Lorz et al. (1979) also discussed a large fishkill that occurred in the Rogue River in Oregon, which was apparently caused by the premature release of acrolein-treated irrigation water. The water in an irrigation canal, which had been treated for aquatic plant control with a gaseous form of acrolein (Magnecide H), was released into the Rogue River after 1 day instead of the recommended 6 days. For a 10-mile portion of the Rogue River, an estimated 238,000 fish were killed. No other details of this spill were given.

3.7 EFFECTS ON TERRESTRIAL LIFE

Although it is released primarily to the aquatic environment, acrolein contamination of agricultural lands and other terrestrial environments is possible as a result of irrigation. Although such contamination was identified as a concern over 20 years ago, actual studies of this phenomenon were not found in the literature. Aside from information on laboratory animals (see chapter 6), available information on the effects of acrolein on terrestrial life is limited to a single laboratory study of one higher plant, the lily.

3.7.1 Terrestrial Plants

Studies on the toxicity of acrolein to terrestrial plants were found for only one species, Lilium longflorum (a lily). Masaru et al. (1976) measured inhibition of pollen tube elongation in lily pollen grains exposed to vapors of acrolein (up to 1.7 ppm) or four other gases. Pollen harvested from lily plants and grown in a greenhouse in activated carbon-filtered air were stored at -10 °C before testing began. Pollen was then sown on petri plates (1 percent agar, 10 percent sucrose, and 100 ppm boric acid) and placed in a fumigation chamber providing standardized growth conditions (28 °C, 60 percent humidity). Pollen tube length was measured in control plates and test plates exposed to acrolein at 0.4, 1.4, and 1.7 ppm for 1, 2, or 5 hours. Test vapors were generated by bubbling air through acrolein solutions; acrolein concentrations were measured by the m-aminophenol method.

Pollen tube elongation was completely inhibited (compared to controls) in pollen grains exposed to acrolein at 0.4 ppm for 5 hours, 1.3 ppm for 2 hours, and 1.7 ppm for 1 hour. When exposed at 0.4 ppm for 1 hour, elongation was only reduced by 10 percent.

3.7.2 Terrestrial Animals

The toxic effects of acrolein on laboratory rats, cats, rabbits, and dogs are discussed in chapter 6. No information was found on the compound's effects on the animals of terrestrial ecosystems.

3.8 BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION

The environmental hazards posed by accumulation of many chemicals in the tissues of both aquatic and terrestrial biota are a serious concern. Environmental levels of certain compounds that appear to be safe even in chronic toxicity tests may accumulate to harmful levels in many organisms like fish and birds. Three potentially important accumulation processes include bioconcentration (direct absorption from the water), bioaccumulation (absorption from food and/or water), and biomagnification (absorption through the food chain).

Available data were limited to a study of the bioconcentration of acrolein in bluegill sunfish (Lepomis macrochirus). Veith et al. (1980) reported the bioconcentration factor (BCF) to be 344 with a half-life in tissues of more than 7 days. The fish were exposed to acrolein at 13.1 ± 26 µg/l for 28 days in a modified Mount-Brungs intermittent flow proportional dilutor. ^{14}C-labeled residues in whole fish and water were measured by liquid scintillation after 1, 2, 4, 7, 10, 14, 21, and 28 days of exposure and after 1, 2, 4, and 7 days of depuration. None of the metabolites was identified.

The measured BCF (344) is higher than would be predicted from acrolein's octanol/water partition coefficient (log P), reported to be 1.2 (Veith et al., 1980).

No other information was found on acrolein's bioconcentration in animals or on its bioaccumulation and biomagnification.

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4. ACROLEIN: MAMMALIAN METABOLISM AND KINETICS OF DISPOSITION

4.1 INTRODUCTION

Acrolein (2-propenal, acrylic aldehyde) is a highly reactive aldehyde which is used as a synthetic intermediate in a wide variety of industrial processes. It is also produced in automobile exhaust, tobacco smoke, and as a degradation product during overheating of oils and fats (Izard and Libermann, 1978; reviewed in Chapter 3). Acrolein is also encountered in mammalian systems as a metabolite from the metabolic conversion of a variety of other xenobiotics (Table 4-1).

Acrolein contains a highly reactive, aldehyde-conjugated double bond, with a strong reactivity in particular toward free sulfhydryl groups or thiols; its high reactivity has an important relationship to its toxicity (Izard and Libermann, 1978). Under ambient environmental conditions, acrolein, a volatile liquid with an acrid and pungent odor, has a high vapor pressure of 400 torr (at 34.5°C) and hence inhalation is an important route of entry to the body. It is an irritant and vesicant to the eyes, and to the mucosae of the nose, bronchial tree and lungs. Acrolein is both soluble in water (about 20 percent, w/v) and lipid-soluble, and hence may be expected, on the basis of these physicochemical properties, to diffuse freely across mucosal barriers of the lungs and gastrointestinal tract into the body. Contrariwise, acrolein's propensity to react with free thiol groups may effectively restrict free diffusion across membranes at portals of entry into the body as well as across membranes of body compartments within the organism.

4.2 ABSORPTION

4.2.1 Oral

Acrolein is known to be absorbed into the body from the gastrointestinal tract but the completeness and kinetics of the process have not been quantified. Rats administered acrolein in corn oil solution by gastric intubation (10 mg/kg

TABLE 4-1. XENOBIOTICS BIOTRANSFORMED IN VIVO TO ACROLEIN

Compound	Mechanisms: Experiment Conditions	Investigator
Allyl alcohol	Alcohol dehydrogenase; isolated hepatocytes and renal epithelial cells, rat liver cytosol, rat liver microsomes	Ohno et al., 1985 Patel et al., 1980, 1983 Serafini-Cessi, 1971
Allylamine	Amine oxidase; rat and human tissue homogenates	Boor and Nelson, 1981, 1982
3-Substituted Propylamines	Amine oxidase; cell culture + serum	Kawase et al., 1982
2-Substituted Propyl alcohol	Horse liver alco. dehydrogenase	Alston et al., 1981
Spermine, Spermidine	Amine oxidase; incubation with calf serum	Alarcon, 1964, 1970 Kawase et al., 1982
2,2,2-Trifluoroallyl ether	P450-catalyzed; rat liver microsomes	Murphy et al., 1983
Cyclophosphamide, Isophosphamide	P450-catalyzed; rat liver microsomes	Alarcon and Meienhofer, 1971 Alarcon et al., 1972 Marinello et al., 1984

body weight) have been found to excrete mercapturic acid metabolites in their urine, but at such a low yield that the question arises whether incomplete absorption other unsampled pathways of metabolism was the explanation (Draminski et al., 1983). Fassett (1962) has reported that the acute oral LD_{50} for acrolein in rats is 46 mg/kg. Similar oral LD_{50} values have been reported for the rat (42 mg/kg) and mouse (28 mg/kg) by Albin (1962). These data presume that death was occasioned by systemic toxicity after absorption. Albin (1962) records that rats administered acrolein in drinking water up to 200 ppm for 3 mo (about 4 mg/d ingestion) showed little evidence of toxicity, either in weight gain or pathological changes.

Acrolein has a great propensity to interact with thiol groups to form conjugated compounds, in aqueous solutions at physiological hydrogen ion concentrations (Section 4.4.2). The effect on absorption of acrolein by these or other noncatalytic chemical reactions as induced or modified by the presence of food or other conditions of the gastrointestinal tract has not been investigated but these reactions are likely to have a limiting influence on absorption of the acrolein molecule per se.

4.2.2 Dermal

The acute lethal toxicity of acrolein by the percutaneous route for the rabbit ranges from LD_{50} of 160 to 1000 mg/kg body weight, depending on the vehicle and concentration (Albin, 1962). It can be inferred from these data that acrolein is capable of crossing the dermal barrier by diffusion but further information has not been reported.

4.2.3 Pulmonary

Controlled studies to determine pulmonary uptake rate and absorption of acrolein into the systemic circulation of man or other mammalian species have not been conducted. The respiratory mucosa and lung alveolar endothelia may act as an efficient barrier for low inhaled doses of acrolein because of the reactivity of acrolein with reduced glutathione content of these cells (McNulty et al., 1984; Lam et al., 1985), and the ability of lung tissue to metabolize acrolein (Patel et al., 1980).

The acute lethal toxicity (LC_{50}) of acrolein in air by inhalation route is: rat, 8 ppm, 4 hr; dog, 150 ppm, 30 min; and mouse, 175 ppm, 10 min (Albin, 1962). However, pathological findings were limited principally to the lungs, providing little indication of absorption into the systemic circulation. Similar observations have been reported with subchronic and chronic daily inhalation exposures to rodents (Kutzman et al., 1985; Boulez et al., 1974; Feron et al., 1978; Lyon et al., 1970; Watanabe and Aviado, 1974).

Egle (1972) has investigated the respiratory uptake of acrolein by pentobarbital-anesthetized dogs at air exposure concentrations of 0.4 to 0.6 μg/ml (∿ 175 to 265 ppm) during a complete ventilatory cycle. The inhaled amount of compounds (air concentration times tidal volume) was designed to mimic the content of acrolein in a 40-ml puff of cigarette smoke (∿ 8.2 μg). The percentage of aldehyde taken up by the respiratory tract of the dog was calculated using the measured amount inhaled (or exposed to tissues) and the amount recovered in exhaled air. The retained uptake of acrolein by the total respiratory tract of dogs (nasal and lung mucosa) at ventilatory rates of 6 to 20 respirations per min averaged 80 to 85 percent and was independent of ventilation rate. By tracheal cannulation, uptake and retention by the upper respiratory tract (between nose and bronchioles) was estimated; it was found that only about 20 percent of acrolein inhaled reached the lower tract. Exposure of the lower tract alone (lung mucosa) resulted in about 65 to 70 percent retention of acrolein dose (decreasing slightly with increase of ventilation rate). Generally, the percent retention of acrolein under these experimental conditions was not affected over a 2 to 3-fold concentration range but did decrease slightly (85 percent to 77 percent) with increase of tidal volume (100 ml to 160 ml). These studies do not specifically provide information on the disposition of the retained acrolein, and because of the brevity of exposure they do not provide pertinent information on pulmonary uptake and body disposition for exposure conditions common to the workplace or ambient environment. However, the results indicate that acrolein, in relatively high air concentrations, is rapidly and extensively removed from inhaled air by both the upper and lower respiratory tract, presumably by interacting with thiol groups of mucus secretions or cell surfaces or by diffusion into mucosal and alveolar endothelial cells where the compound may react with cellular sulfhydryl groups, be metabolized, or be absorbed into the body (Section 4.4).

McNulty et al. (1984) and Lam et al. (1985) have exposed rats to acrolein in air (0.1 ppm to 5 ppm) for 3-hr periods and demonstrated a marked decrease of non-protein sulfhydryls (glutathione, GSH) in the respiratory nasal mucosa in a concentration-dependent manner (Figure 4-2). Liver GSH content was also determined by McNulty and coworkers, but as it did not decrease, it did not provide evidence of absorption of acrolein into the systemic circulation for these experimental conditions.

4.3 DISTRIBUTION AND EXCRETION

The distribution of acrolein into body tissues after entry into the body from oral or inhalation exposures has not been experimentally documented. Distribution may be expected to be substantially influenced by the chemical reactivity of acrolein in aqueous solution at physiological hydrogen-ion concentrations of blood and extracellular fluids. After oral administration to rats (10 mg/kg b.w.), mercapturic acid metabolites of acrolein are found in the urine, indicating distribution to the liver and occurrence of hepatic metabolism (Draminski et al., 1983). As a dose-dependent depletion of liver reduced glutathione has been shown to occur with parenteral administration of acrolein (Gurtoo et al., 1981; Patel and Leibman, 1978), a dose-dependent first-pass effect on hepatic clearance after oral exposure can be predicted. Similar considerations pertain to inhalation exposure. After a 3-hr inhalation exposure of 0.1 to 5 ppm acrolein in air, a dose-dependent depletion of glutathione of respiratory mucosa occurs while hepatic glutathione remains unaffected (McNulty et al., 1984; Lam et al., 1985), indicating that pulmonary clearance and distribution to the liver are limited. Blood (plasma and red blood cells) normally contains substantial amounts of reduced glutathione which, acting as a sink for acrolein-glutathione noncatalytic interactions (Section 4.4.2), may limit acrolein distribution into body tissues, particularly under conditions of low exposure concentration.

Virtually nothing is known of the elimination or excretion of assimilated acrolein from the body following oral or inhalation exposures. Of the three major routes of elimination of xenobiotics from the body--renal, pulmonary, and metabolic--acrolein has not been found in urine or exhaled breath after oral or parenteral administration (Draminski et al., 1983; Alarcon, 1976; Kaye, 1973). Hence the principal route of elimination appears to be by metabolism (Section 4.4). However, quantitative studies designed to determine the disposition of acrolein have not been carried out after either acute or chronic dosage. Moreover, blood concentration-time disappearance curves after acrolein dosage have not been reported, and the kinetics of elimination of acrolein from the systemic circulation or from the body remain to be determined.

4.4 METABOLISM

4.4.1 Quantitation of Metabolism

The extent to which a given dose of acrolein is metabolized by mammalian species has not been defined experimentally. Balance studies with labeled or nonlabeled acrolein administered by any route have not been reported. Hence dose-metabolism relationships for acrolein are not available.

Table 4-2 gives the known metabolites of acrolein. Of those listed, only the mercapturic acids S-(hydroxypropyl), S-(carboxyethyl), and S-(propionic acid methyl ester) have been identified in urine after oral, subcutaneous or intraperitoneal administration to rats with a yield of 10 to 18 percent of the given dose (Alarcon, 1976; Kaye, 1973; Draminski, 1983). Kaye and Young (1974)

TABLE 4-2. DEMONSTRATED BIO-METABOLITES OF ACROLEIN

Metabolite	System	Investigator
S-(hydroxypropyl)-mercapturic acid	Rat urine	Kaye, 1973 Alarcon, 1976
S-(carboxyethyl)-mercapturic acid	Rat urine	Draminski et al., 1983
S-(proprionic acid methyl ester) - mercapturic acid	Rat urine	Draminski et al., 1983
Acrylic acid	Rat liver cytosol	Patel et al., 1980
Glycidaldehyde	Rat liver and lung microsomes	Patel et al., 1980
Glyceraldehyde	Rat liver and lung cytosol	Patel et al., 1980

and Alarcon (1976) found significant amounts of S-(hydroxypropyl)-mercapturic acid in human urine after administration of cyclophosphamide, of which acrolein is a metabolite. This observation indicates that the mercapturic acid pathway for acrolein metabolism is not unique to the rodent. Thus at least 80 percent of an administered dose of acrolein remains unaccounted for. Draminski et al. (1983) trapped expired air of rats through a charcoal tube after oral administration of acrolein (10 mg/kg body weight). GC analysis of the expired air on Carbowax 20 M-Gas Chromosorb Q revealed the presence of a volatile compound with a retention time (2.37 min), shorter than acrolein (4.1 min), or the corresponding alcohol or acid: allyl alcohol (14.9 min), or methylacrylate (6.7 min). This fragmentary evidence suggests a portion of the acrolein dose may be excreted via the pulmonary route in the form of a metabolite, but apparently not as acrolein itself. There is, however, sufficient reason to believe, until more complete and satisfactory experimental evidence is available, that acrolein is extensively, if not completely, biotransformed by all mammalian species at exposure doses likely to be encountered in the workplace or ambient environment. As described below (Section 4.4.2), the facile reactivity of acrolein, both noncatalytic and catalytic, in biological systems provides a potential for extensive biotransformation. It may be noted that acrolein has been demonstrated to be biotransformed by hepatic metabolizing systems to acrylic acid and to glyceraldehyde (Table 4-2). Acrylic acid may be incorporated into many endogenous components, including amino acids, fatty acids and sterols, via the formation of acrylyl CoA by thiokinase, and hence to propionyl CoA and beta-hydroxypropionyl CoA. In turn, glyceraldehyde is known to readily enter cellular glycolytic pathways. It is likely, therefore, that a large portion of a given dose of acrolein may eventually be accounted for experimentally by expired CO_2 and/or by incorporation into normal body constituents.

4.4.2 Noncatalytic Interaction with Sulfhydryl Groups

Acrolein, with a conjugated double bond system and a tendency for its beta carbon group to be positively charged, has a high chemical reactivity to reduced sulfhydryl groups (-SH), or thiols at its beta carbon double bond reactive site (Izard and Libermann, 1978). Acrolein has the potential to react with these groups in vivo nonenzymatically as well as enzymatically.

Alarcon (1976) investigated the conjugation of acrolein in phosphate buffer (at pH 7, 7.4, and 8.5, at 23°C) with glutathione (GSH), acetylcysteine and cysteine by measuring the decrease of acrolein's characteristic UV absorbance at 209 nm. With equimolar amounts of acrolein and the sulfhydryl compounds, adduct formation occurred rapidly (dependent on pH), with 50 percent formation at pH 7.4 within 0.5 min (cysteine), 1.0 min (glutathione), and 3.0 min (acetylcysteine). Iodometric titration of the sulfhydryl compounds, before and after interaction with acrolein, confirmed that the reaction involved the sulfhydryl anion in these compounds. Furthermore, the adduct could be decomposed by incubation at 100°C with regeneration of acrolein in high yield. With borohydride the adducts were reduced and then chromatographed. Acrolein-acetylcysteine and acrolein-cysteine adducts yielded on reduction 3-hydroxy-propyl mercapturic acid and 3-hydroxypropyl cysteine, respectively. Alarcon (1976) notes that 3-hydroxypropyl mercapturic acid was isolated by him from rat urine after the administration of acrolein subcutaneously, confirming a prior observation of Kaye (1973).

Esterbauer et al. (1975, 1976) also investigated the kinetics of nonenzymatic interaction of conjugated carbonyl compounds in aqueous solution with glutathione and cysteine and found that acrolein reacted more rapidly than other carbonyls to give very stable adducts. Similar observations have been made by Patel et al. (1984) and Dore and Montaldo (1984), who observed also that acrolein very rapidly conjugates with equimolar amounts of glutathione in water or buffered solutions. The conjugate(s), however, were not isolated and identified by these investigators. Gray and Barnsley (1971) have reported rapid first-order reaction of crotonaldehyde (methylacrolein) with glutathione in aqueous solution (pH 7.5) that results in one major product upon chromatographing. Ohno et al. (1985) has suggested the possibility that glutathione-acrolein may also produce a thiohemiacetal adduct. Such an adduct may be reversible and form a reservoir for release of acrolein for conversion by cellular aldehyde dehydrogenase to form acrylic acid, a known metabolite of acrolein (Patel et al., 1980). The nonenzymatic reactions of aldehydes with glutathione have been reviewed by Ketterer (1982).

Gurtoo et al. (1981) have demonstrated that acrolein (and cyclophosphamide, of which acrolein is a metabolite) injected intraperitoneally into mice produces a dose-dependent depletion of liver glutathione (Figure 4-1). However, fairly high doses (500-1000 mg/kg) were required to deplete cellular glutathione to the 30 percent level. Peak depletion occurred between 2 and 8 hr after injection with a return towards normal levels after 24 hr. Patel and Liebmann (1978) have observed a similar glutathione depletion in rat liver and lung after i.p. injection of acrolein. Respiratory mucosal glutathione depletion in rats has also been observed after inhalation of acrolein (McNulty, 1984). These workers exposed rats to 0.1 to 5 ppm acrolein in air for 3 hr and found a dose-dependent depletion of cellular glutathione (63 percent, at 5 ppm) of nasal mucosa, even though liver glutathione did not decrease with this exposure. Similar results have been demonstrated by Lam et al. (1985), who exposed rats to 0.1 to 2.5 ppm for 3 hr and observed a concentration-dependent decrease in glutathione content of the nasal respiratory mucosa. Their results are shown in Figure 4-2.

Acrolein added to isolated rat hepatocytes (0.025 to 0.25 mM) induced a rapid (10 to 30 min), dose-dependent decrease of cellular glutathione (Zitting and Heinonen, 1980; Dawson et al., 1984). At the highest concentration (0.25 mM), the depletion was virtually complete; at low concentrations recovery of cellular glutathione occurred gradually over a 2-hr period (Zitting and Heinonen, 1980). Similar observations with isolated rat hepatocytes have also been observed with allyl alcohol, the alcohol corresponding to acrolein and biotransformed by alcohol dehydrogenase to acrolein (Table 4-1). Ohno et al.

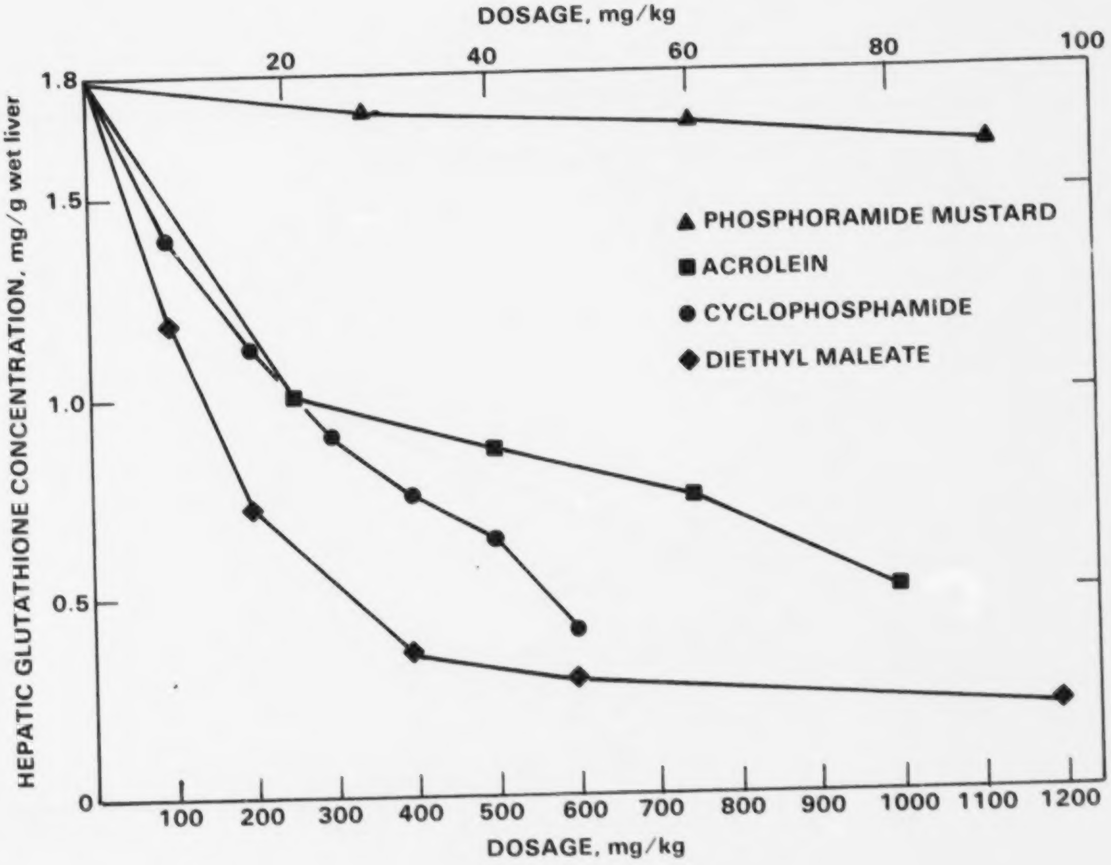

Figure 4-1. Dose-related depletion of liver reduced glutathione (GSH) in mice after intraperitoneal injections of acrolein and cyclophosphamide (acrolein, a metabolite), and of the classic depletor, diethyl maleate. The mice were sacrificed 2 hr after injection.

Source: Gurtoo et al. (1981).

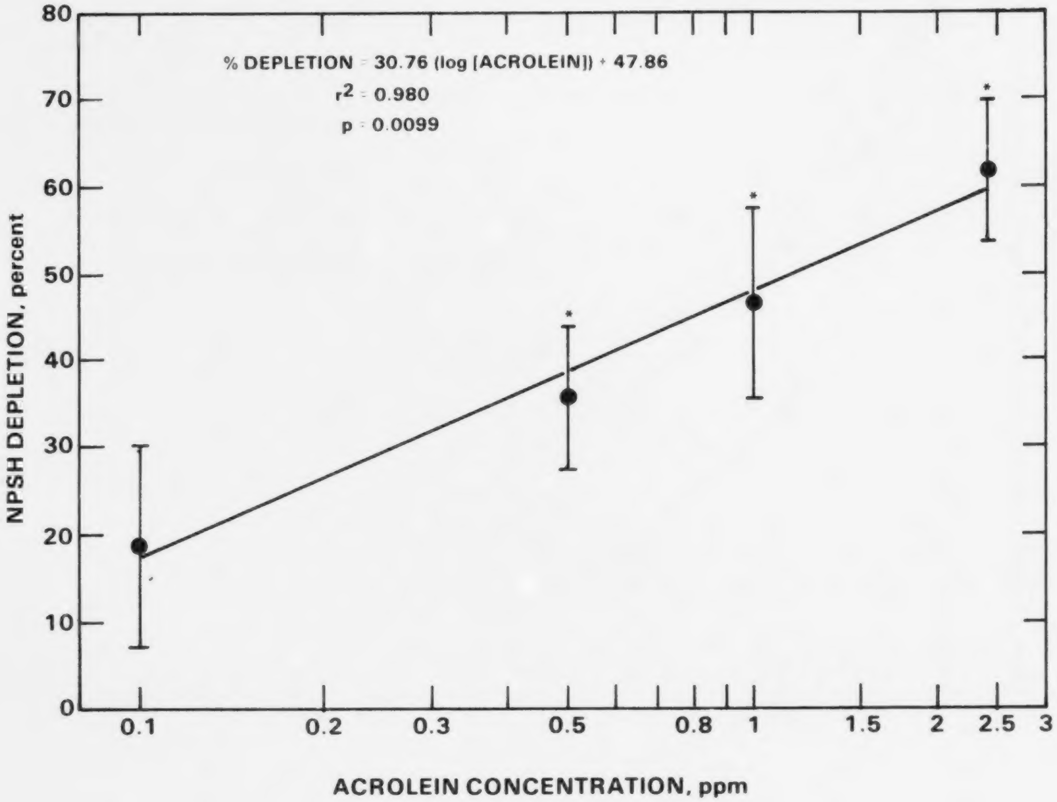

Figure 4-2. Log-dose relationship of percentage depletion of nonprotein sulfhydryl groups (glutathione) in the nasal respiratory mucosa of rats exposed to acrolein for 3 hr. Each data point is the mean of 4 rats ± SE; *denotes $p < 0.05$ compared to controls.

Source: Lam et al. (1985).

(1985) found a rapid (10 to 30 min), dose-dependent decrease of cellular content of glutathione with addition of 0.1 to 0.5 mM allyl alcohol. Depletion was blocked by inhibitors of alcohol dehydrogenase (pyrazole or methylpyrazole). These workers also observed the appearance of acrolein-GSH adducts in the incubation medium, which, however, were not identified chemically. Kaye (1973), who found considerable urinary excretion of mercapturates in rats given allyl alcohol or acrolein subcutaneously, suggested these adducts arise enzymatically from acrolein-glutathione transferase reactions. Boyland and Chasseaud (1967) have demonstrated enzyme-catalyzed conjugations of glutathione with alpha, beta-unsaturated aldehydes including acrolein-diethylacetal and crotonaldehyde, a homolog of acrolein.

The observations reviewed above impart considerable uncertainty as to the extent acrolein reactions with glutathione and other free sulfhydryls _in vivo_ occur catalytically or noncatalytically (or both). The glutathione transferases are ubiquitous in vivo, although the evident high reactivity of acrolein with glutathione and other free sulfhydryls in aqueous solutions at physiological pH and temperature increases the likelihood that some of these interactions are not glutathione-transferase-dependent. The further _in vivo_ conversion of S-substituted glutathione, whether from enzymatic or nonenzymatic origins, to the corresponding mercapturic acids is well understood and documented (Arias and Jakoby, 1976).

4.4.3 Enzymatic Pathways

Acrolein appears to undergo biotransformation via two major pathways: 1) oxidation to acrylic acid and/or conjugations with glutathione; 2) oxidation to glycidaldehyde and conjugations with glutathione. Figure 4-3 illustrates these pathways and their interrelationships. The evidence derives primarily from _in vitro_ experiments with liver and lung tissue preparations and from metabolite identification in urine; no well-designed _in vivo_ balance studies with labeled acrolein have been conducted. Consequently the relative importance of the pathways has not been clearly assessed.

Acrylate pathway: Patel et al. (1980) have demonstrated the conversion of acrolein to acrylic acid by rat liver (9000 g supernatant fraction, cytosolic fractions or microsomes fortified with either NAD+ or NADP+). About 20 percent and 7 percent of added acrolein was metabolized to acrylic acid by cytosol fractions by these NAD+ and NADP+ dependent aldehyde dehydrogenase reactions in cytosolic fractions respectively. Disulfiram (at 0.5 and 1.0 mM) completely

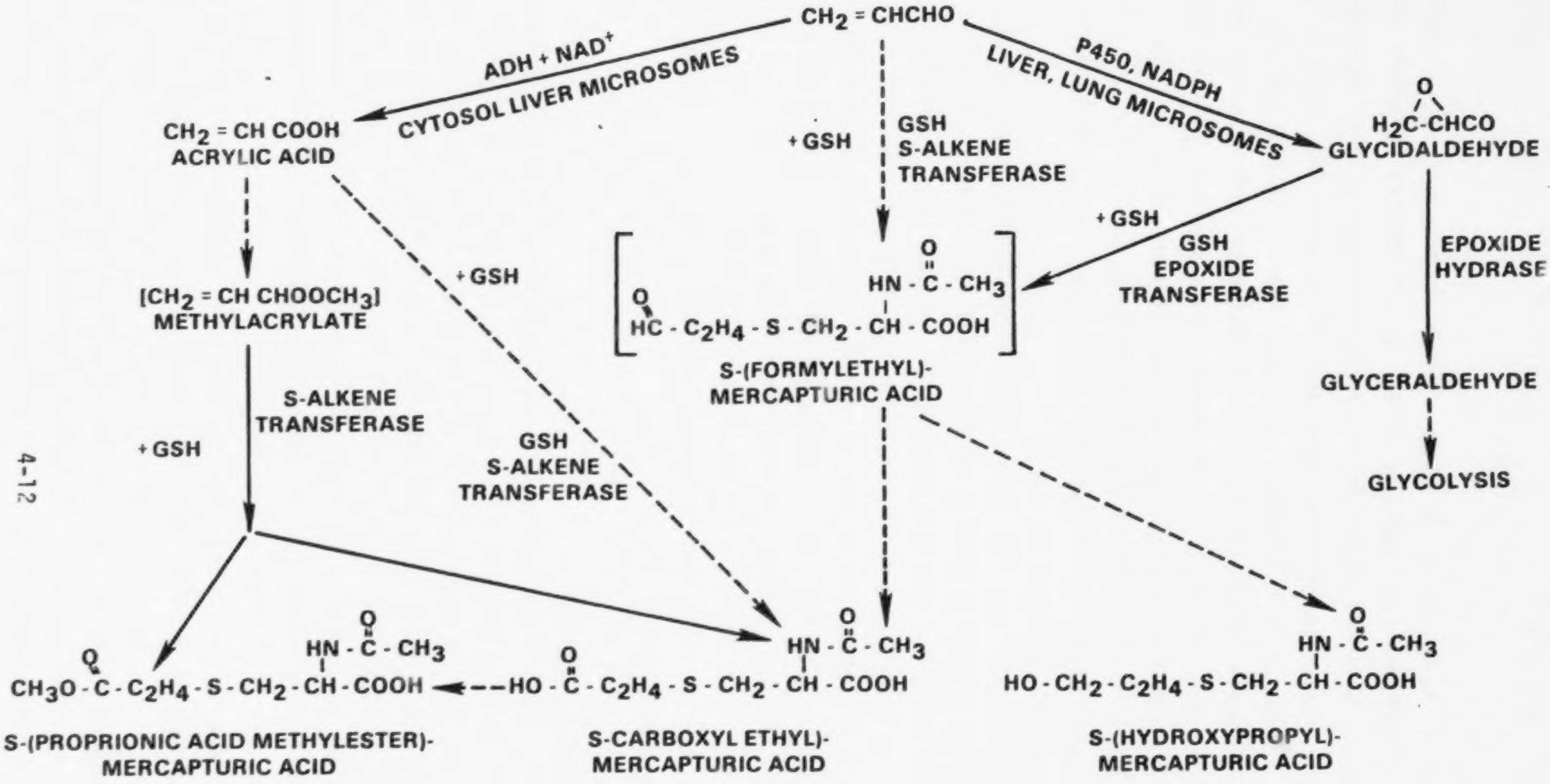
CH2 = CHCHO
ADH + NAD+
CYTOSOL LIVER MICROSOMES
P450, NADPH
LIVER, LUNG MICROSOMES
CH2 = CH COOH
ACRYLIC ACID
+GSH
GSH
S-ALKENE
TRANSFERASE
O
H2C-CHCO
GLYCIDALDEHYDE
+GSH
GSH
EPOXIDE
TRANSFERASE
EPOXIDE
HYDRASE
[CH2 = CH CHOOCH3]
METHYLACRYLATE
+GSH
HC - C2H4 - S - CH2 - CH - COOH
HN - C - CH3
S-(FORMYLETHYL)-
MERCAPTURIC ACID
GLYCERALDEHYDE
GLYCOLYSIS
S-ALKENE
TRANSFERASE
+GSH
GSH
S-ALKENE
TRANSFERASE
CH3O - C - C2H4 - S - CH2 - CH - COOH
HN - C - CH3
S-(PROPRIONIC ACID METHYLESTER)-
MERCAPTURIC ACID
HO - C - C2H4 - S - CH2 - CH - COOH
HN - C - CH3
S-CARBOXYL ETHYL)-
MERCAPTURIC ACID
HO - CH2 - C2H4 - S - CH2 - CH - COOH
HN - C - CH3
S-(HYDROXYPROPYL)-
MERCAPTURIC ACID

Figure 4-3. Postulated pathways of acrolein metabolism. The three mercapturic acids have been found in rat urine after acrolein administration; the solid arrows represent demonstrated reactions (see text).

Source: Modified after Patel et al. (1980); Draminski et al. (1983).

inhibited acrylic acid formation by 9000 g supernatant fractions, cytosolic fractions, and by microsomes indicating reaction dependence on aldehyde dehydrogenase. Similar fractions from rat lung did not form acrylic acid, presumably because of the absence of both alcohol and aldehyde dehydrogenase activities in this tissue (Patel and Leibman, 1978). [Acrolein is also a substrate for human, horse and yeast alcohol dehydrogenase, although for the pair 2-propenol: 2-propenal, the equilibrium constant favors the conversion of alcohol to aldehyde (Pietruszko et al., 1973)].

Draminski et al. (1983) isolated S-(proprionic methyl ester) mercapturic acid in rat urine after oral dosing with 120 mg acrolein/kg. Since, however, the urine sample was methylated for GC-MS analysis, it was not possible to exclude the non-methyl ester S-carboxylethyl mercapturic acid as the metabolite (or both). The acrylic acid ester, methyl acrylate, when administered to rats, has been shown to yield in rat urine S-carboxylethyl mercapturic acid and its nonmethyl ester, S-(proprionic acid methyl ester) mercapturic acid in a ratio 20:1 (Delbressine et al., 1981). Thus Draminski et al. suggest that acrylic acid formed from acrolein is esterified to methyl acrylate which then conjugates with glutathione catalyzed by glutathione S-alkenetransferase as described by Boyland and Chasseaud (1968). Since there is uncertainty as to the complete substrate specificity of glutathione S-alkene transferase(s) (Boyland and Chasseaud, 1968; Delbressine et al., 1981; Kaye, 1973), the possibility remains that acrylic acid and acrolein themselves may also serve as substrates for these transferases. Kaye (1973) and also Alarcon (1976) have shown that rats injected subcutaneously or intraperitoneally with acrolein (16 to 2,500 mg/kg) excreted in the urine 10 percent to 18 percent of the dose (increasing with dose) as S-(hydroxypropyl)-mercapturic acid. Kaye suggested that this metabolite was likely the result of acrolein conjugation with glutathione catalyzed by one of the many glutathione S-alkene transferases described by Boyland and Chasseaud (1967, 1968). In fact, Boyland and Chasseaud (1967) have demonstrated that acrylic acid esters, acrylic acid homologs and acrolein diethylacetal and acrolein homologs are substrates for these transferases. At some stage during the conversion of the glutathione conjugate into the mercapturic acid, reduction of the carbonyl moiety to an alcohol group must occur as well as peptidase conversion of the conjugate to the mercapturic acid.

Glycidaldehyde pathway: Patel et al. (1980) have demonstrated with both rat liver and lung microsome preparations fortified with NADPH the formation of the epoxide of acrolein, glycidaldehyde and the hydration of the epoxide to

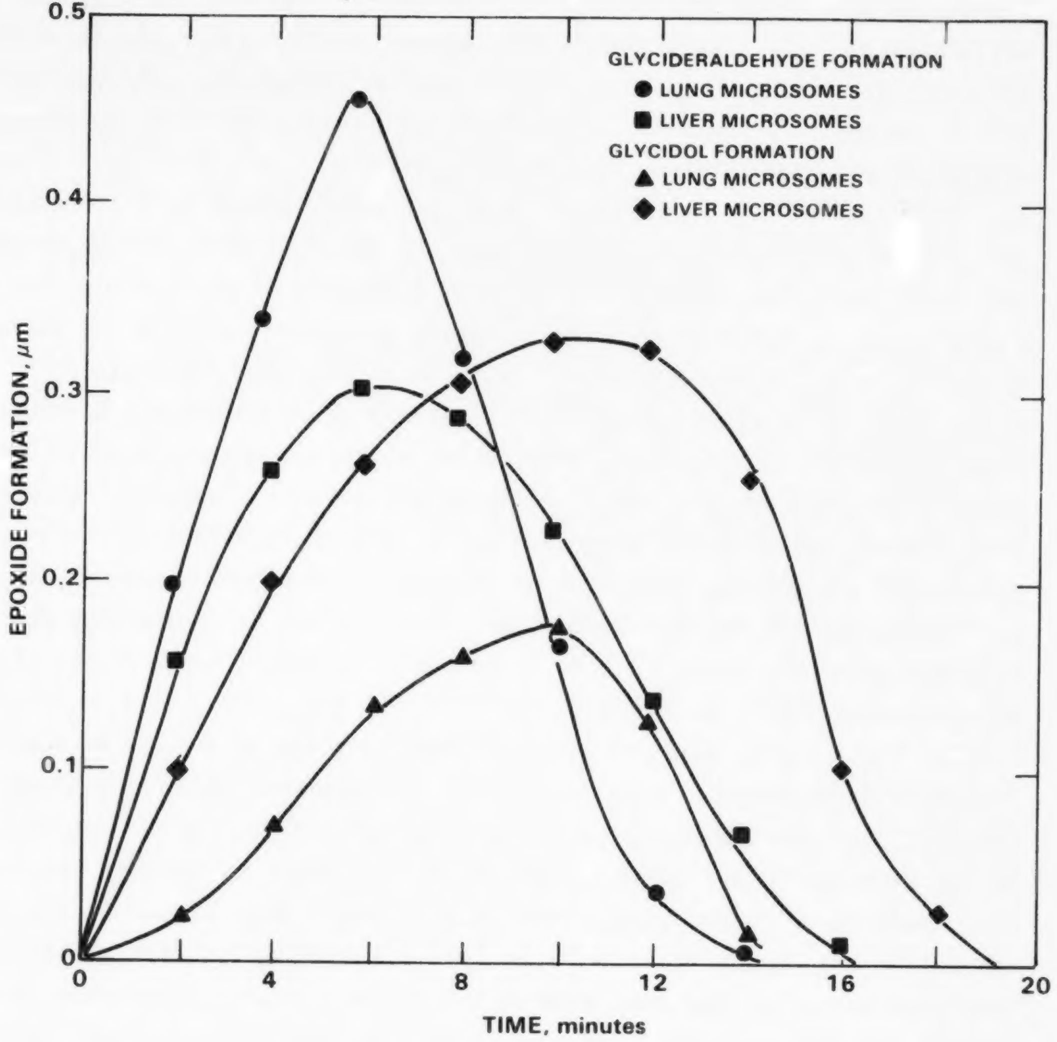

Figure 4-4. Rate of formation of epoxide from acrolein and from allyl alcohol by microsomes isolated from rat liver or lung.

Source: Patel et al. (1980).

glyceraldehyde. Figure 4-4 shows the time course of epoxidation of acrolein (and of the corresponding alcohol, allyl alcohol) by rat liver and lung microsomes. The apparent decline in rate of production of the epoxide after about 6 min incubation can be accounted for by hydration to glyceraldehyde (by epoxide hydratase) and by inactivation of microsomal cytochrome C reductase and conversion of P450 to P420 by acrolein or its epoxide at a similar time course (Patel et al., 1980, 1984; Marinello et al., 1981). Glycidaldehyde was also found by Patel et al. (1980) to be a substrate for both rat liver and lung cytosolic activity presumed to be glutathione epoxide transferase(s). Fjellstedt et al. (1973) previously have shown that glycidaldehyde was a substrate for purified glutathione epoxide transferase(s) isolated from rat liver cytosol. While the structure of the product of the enzymatic reaction of glutathione and the epoxide was not rigorously established, evidence was obtained to suggest that the product was an unsymmetrical thioether between glutathione and one of the oxirane carbons. These observations indicate that glycidaldehyde conjugates with glutathione with subsequent conversion to a mercapturic acid, possibly S-(hydroxypropyl)-mercapturic acid or S-(carboxyethyl)-mercapturic acid, which are found in rat urine (Table 4-2; Figure 4-3). Glyceraldehyde, the hydration product of glycidaldehyde, may be expected to enter the normal cellular energy pathways by conversion to glyceraldehyde-3 phosphate by the enzyme ATP: D-glyceraldehyde 3-phosphate transferase [E.C.2.T.1.28] and thereby enter the glycolytic pathway to pyruvate or lactate. This may also represent a principal pathway for acrolein biotransformation.

4.4.4 Covalent Binding

Reactivity to nucleic acid in solution: Acrolein and other alpha, beta-unsaturated carbonyl compounds such as crotonaldehyde can interact directly, not only with free thiols (Section 4.4.2), but also with nucleic acids (Izard and Libermann, 1978). Descroix et al. (1971) have found that even in aqueous solution *in vitro*, acrolein reacts with nucleotides and DNA, using as a measure of reaction the disappearance of the UV spectra of acrolein. Munsch et al. (1974) interacted ^{3}H-acrolein (0.6 mM) in tris buffer (pH 7.0; 38°C) with calf thymus DNA and synthetic nucleotide polymers and found covalent binding of radioactivity in the ratio of 1 to 2 molecules of acrolein per 1000 nucleotide units. Recently, Chung et al. (1984) have demonstrated direct adduct formation from acrolein-deoxyguanosine and acrolein-calf thymus DNA interactions in phosphate buffer at pH 7, 37°C. These investigators identified cyclic 1,N^2-propanodeoxyguanosine adducts from these interactions, with a possibility of other adducts with other DNA bases or cross-linking adducts. Glycidaldehyde, the bifunctional epoxide metabolite of acrolein (Figure 4-3), has also been shown to react with guanosine *in vitro* to form a cyclic derivative between N^1 and N^2 (Goldschmidt et al., 1978).

Binding in biological systems *in vitro* and *in vivo*: Lam et al. (1985) have reported evidence that acrolein can interact with nucleoprotein (like formaldehyde; Ross and Shipley, 1980; Casanova-Schmitz et al., 1984) to produce "DNA-protein cross-links" as a result of covalent binding. Cross-linking of DNA to proteins causes a decrease in the extractability of DNA from tissue proteins. The "absent" DNA can be quantitatively recovered from the proteins after proteolytic digestion. Acrolein was added to rat respiratory mucosa tissue-homogenates *in vitro* (0°C, 10 min incubation) and subsequently DNA was isolated by extraction with chloroform/iso-amyl alcohol/phenol (14/1/25) solvent mixture followed by centrifuging. DNA in the aqueous and interface layers was determined before and after digestion with proteinase K. Table 4-3 shows a concentration-dependent increase in the percentage of interfacial layer DNA, indicating the formation of DNA-protein cross-links. However, similar experiments with respiratory mucosa taken from rats exposed to acrolein (2 ppm for 6 hr) *in vivo* revealed no significant increase in the percent interfacial DNA in comparison with unexposed rats. Thus, acrolein appeared to form few, if any, DNA-protein cross-links with *in vivo* exposure conditions. Acrolein did, however, enhance DNA-protein cross-links *in vivo* from formaldehyde exposure, presumably by causing glutathione depletion and thereby decreasing potential for formaldehyde glutathione detoxification pathway.

Munsch et al. (1974) have investigated the *in vivo* binding of ^{3}H-acrolein to nucleic acids and protein in the 3-day partial hepatectomized rat model of liver regeneration. After intraperitoneal injections, partition of radioactivity of ^{3}H-acrolein in the liver cellular fractions--acid-soluble, lipids, proteins, RNA and DNA--was approximately 93, 3.5, 1.25, 0.65 and 0.35 percent respectively of total liver radioactivity, with little change in distribution percentage at timed intervals 10 min to 24 hr. These results suggest rapid (within 10 min) and stable binding to cellular macromolecules. Table 4-4 shows the time course of the binding of ^{3}H-acrolein to nucleic acid and protein isolated from liver. The binding of radioactivity to these macromolecules is

TABLE 4-3. DNA-PROTEIN CROSS-LINKING: QUANTITIES OF DNA RECOVERED IN THE AQUEOUS PHASE AND IN THE AQUEOUS-ORGANIC INTERFACE OF RAT NASAL MUCOSAL HOMOGENATES INCUBATED WITH SELECTED CONCENTRATIONS OF ACROLEIN*

Acrolein Concentration (mM)	DNA (mg/g tissue)		Interfacial DNA (%)
	Aqueous	Interface	
0	4.69	0.48	9.3
0.3	4.14	0.43	9.4
3.0	4.45	0.69	13.4
30.0	3.64	1.26	25.7

*Each homogenate (in 0.1 M phosphate containing 5 mM EDTA pH 8) was prepared from the respiratory mucosa of 3 rats and was incubated with acrolein at the indicated concentration for 10 min (0°C). Aqueous and interfacial DNA were isolated by extraction with a phenolic solvent system and protein digestion.

Source: Lam et al. (1985).

TABLE 4-4. COVALENT BINDING OF ^{3}H-ACROLEIN* TO RNA, DNA AND PROTEIN OF REGENERATING RAT LIVER

Time After Injection	Radioactivity--Acrolein Equivalents		
	Protein p mol/mg	RNA p mol/μg P	DNA p mol/μg P
10 min	4.57**	0.6	0.7
30 min	5.85	0.48	0.85
1 hr	6.0	0.48	0.77
3 hr	7.6	0.48	0.67
5 hr	7.02	0.48	0.95
24 hr	7.75	0.48	0.60

*^{3}H-acrolein injected intraperitoneally into male Wistar rats, 70 hr after partial hepatectomy (75 mCi/m mole).
**Mean of 4 animals.

Source: Munsch et al. (1974).

rapid, 80 to 90 percent occurring within 10 min after injection of ^{3}H-acrolein. The binding to protein is augmented significantly during the first hour following injection, but the binding to DNA and RNA is not significantly increased with time. This may suggest that the acrolein binding to nucleic acids is in large part direct, i. e. nonenzymatic, and does not require prior metabolism.

Munsch and Frayssinet (1971) have shown that acrolein (50 to 270 μg/100 gm; i.p.), in a dose-dependent manner, strongly inhibits the de novo synthesis of DNA and RNA in the liver and lungs of partially hepatectomized rats, as measured by labeled thymidine, orotic acid and ^{32}P incorporation. Previously, Leuchtenberger et al. (1968) had observed that acrolein inhibited RNA synthesis by mouse-kidney epithelial tissue in cell culture. Investigations of this phenomenon by Munsch and associates (Moulé and Frayssinet, 1971; Munsch et al., 1973, 1974) indicate that acrolein inhibits RNA polymerase transcription by acting on the enzyme itself rather than on the DNA template. Moulé and Frayssinet (1971) demonstrated that the addition of acrolein to isolated rat liver nuclei in tris buffer in vitro inhibited in a dose-dependent manner RNA polymerase-dependent polymerization of ATP, UTP and GTP. Inhibition was independent of added calf thymus DNA, whereas addition of progressively higher levels of RNA polymerase enzyme led to a partial recovery. Munsch and Frayssinet (1973) studied also the effects of acrolein on DNA synthesis in vitro. These investigators used two different DNA polymerases: regenerating rat liver DNA polymerase and E. coli DNA polymerase I, with templates of calf thymus DNA or linear polymers of adenine-thymidine nucleotides. Acrolein produced an inhibitory effect on rat DNA polymerase activity at concentrations above 8×10^{-5}M (although an activation effect occurred at low concentrations). In the presence of 2-mercaptoethanol, acrolein inhibitory effect was suppressed and enzyme activity restored. Hence these workers postulated that acrolein, by interacting with the active thiol groups of rat DNA polymerase, inhibits enzyme activity. This hypothesis was consistent with the observation that E. coli enzyme devoid of SH groups in its active center was not inhibited by acrolein. Munsch et al. (1974) further explored the effect of acrolein on rat liver DNA polymerase through binding studies with ^{3}H-acrolein. When DNA polymerase was incubated in vitro with ^{3}H-acrolein (6×10^{-4}M), binding to the enzyme increased linearly with ^{3}H-acrolein concentration. Binding to enzyme preincubated with mercaptoethanol was blocked, and addition of mercaptoethanol after binding occurred could not reverse the covalent binding. Binding of ^{3}H acrolein to E. coli DNA polymerase also occurred but was 10 to 20 times less than that with rat liver DNA polymerase. Binding also occurred to calf thymus DNA or synthetic nucleotide polymer templates but at higher concentrations (6 x 10^{-4}M). These investigators suggest that acrolein has a greater reactive affinity for rat liver DNA polymerase sulfhydryl groups than to DNA nucleotide binding.

Marinello et al. (1984) studied the binding of ^{14}C-acrolein to rat hepatic microsomes, and the effect of glutathione and other free sulfhydryl and amino groups on the binding. The reaction was carried out in the absence of coenzyme (NADPH) so that binding was not mediated by metabolism. Table 4-5 shows that in the absence of metabolism significant binding occurred. Glutathione, cysteine and acetylcysteine effectively blocked the binding which can therefore be assumed to be primarily with microsomal sulfhydryl groups. The amino group in lysine was only marginally effective in blocking binding. Table 4-6 shows the results, with added coenzyme, of metabolism-mediated binding. While ^{14}C-acrolein binding per se in the absence of metabolism showed significant binding to the microsomes (Table 4-5), the binding was substantially enhanced in the presence of NADPH, suggesting strongly that a metabolite of acrolein (probably

TABLE 4-5. BINDING OF ^{14}C-ACROLEIN TO HEPATIC MICROSOMES FROM PHENOBARBITAL-TREATED RATS IN THE ABSENCE OF NADPH

Treatment	^{14}C-acrolein bound, nmol/mg protein
None	-
Acrolein (4mM)	37
	As % of ^{14}C-acrolein bound
Acrolein (4mM)	100
Plus Glutathione	10
Plus Cysteine	18
Plus N-acetylcysteine	28
Plus Lysine	88

Source: Marinello et al. (1984).

TABLE 4-6. METABOLISM-MEDIATED BINDING OF ^{14}C-ACROLEIN TO HEPATIC MICROSOMES PHENOBARBITAL-TREATED RATS; NADPH ADDED

Treatment	Radioactivity as p mol acrolein/mg protein	
	Exp. 1	Exp. 2
With added NADPH minus without NADPH	2707	5700
With added NADPH minus without NADPH plus SKF 525A	6	<6
Control microsomes (saline treated); with NADPH minus NADPH	23	<10

Source: Marinello et al. (1984).

glycidaldehyde) was also binding to the microsomes. The enhancement of binding is clearly metabolism-dependent since SKF-525A completely abolished the difference in binding observed between plus and minus NADPH. In addition, compared to phenobarbital-treated microsomes, noninduced control microsomes generated considerably less metabolite binding (Table 4-5).

Patel et al. (1984), Gurtoo et al. (1981, 1983), Ivanetich et al. (1978) and Marinello et al. (1981) have demonstrated total destruction of liver microsomal NADPH-cytochrome C reductase activity and reduction of P450 cytochrome activity by acrolein covalent interaction and binding to free cysteine sulfhydryl groups including those in or near the active sites of these cytochromes. Experiments *in vitro* with purified cytochromes and liver microsomes indicate that acrolein inactivates these enzymes by forming adducts with sulfhydryl groups in their protein structures by direct, non-catalytic interaction. Microsomal protein-bound sulfhydryl groups were decreased upon incubation with acrolein, while addition of chemicals containing a free sulfhydryl group (glutathione, dithiothreitol, N-acetylcysteine, cysteine) prior to the addition of acrolein provided significant protection of cytochrome activity.

4.5 SUMMARY

Acrolein, CH_2=CHCHO, is a highly reactive aldehyde which reacts nonenzymatically and enzymatically to 1) form stable adducts with extracellular and intracellular glutathione and other free thiol groups, 2) form adducts with nucleic acids and proteins, 3) cross-links nucleic acids and proteins, and 4) reacts with enzymes and membranes to cause a variety of biochemical consequences such as impairment of DNA replication, inhibition of protein synthesis and mitochondrial respiration, loss of liver and lung microsomal enzyme activities, and other parameters of cellular integrity. This propensity for covalent binding by acrolein provides the basis for its cellular toxicity.

The reactivity and toxicity of acrolein is manifested in the gastrointestinal and pulmonary tracts, the principal sites of exposure. There is no documented evidence (for instance, blood determinations) that for exposure concentrations below aversive levels, acrolein breaches the protective mechanisms at these portals to gain entry to the general circulation. Bronchial and mucosal secretions, and mucosal and endothelial tissues at these locations contain high concentrations of free thiols. Lung, gastrointestinal mucosa and liver also contain effective metabolizing systems with high capacity to biotransform acrolein. Hence the extent and kinetics of absorption of acrolein into the body during oral and inhalation exposure remain to be determined.

Acrolein can be formed in vivo via the metabolism of a number of xenobiotics. For example, allyl alcohol, CH_2CHCH_2OH, causes intensive hepatic periportal necrosis in rats after oxidation by hepatic alcohol dehydrogenase to acrolein. Since protection from allyl alcohol toxicity is afforded by pretreatment with sulfhydryl group donors (cysteine or N-acetylcysteine), it is presumed that when free thiol groups are depleted by acrolein interaction, acrolein combines covalently with other nucleophilic groups of cellular macromolecules and thus leads to cellular damage.

Acrolein has been demonstrated to be metabolized in vivo, particularly by liver and lung parenchymal tissues. Two major pathways have been demonstrated: (1) nonenzymatic and/or glutathione transferase reactions to form stable adducts with glutathione and other thiols leading to mercapturic acid metabolites, and (2) oxidative metabolism to (a) acrylic acid via aldehyde dehydrogenase activity, and (b) to the epoxide glycidaldehyde via microsomal P450 oxidation system. Glycidaldehyde, a reactive metabolite capable of covalent binding, is further metabolized to innocuous metabolites by cellular glutathione epoxide transferase or by epoxide hydrase. The relative importance of these various pathways has not been assessed, nor has the effect of acrolein dosage on metabolic disposition. However, acrolein appears to be extensively, if not completely, metabolized in mammalian systems; acrolein itself has not been found in urine or exhaled air of rodents after parenteral administration of high doses. Hence metabolism appears to be the principal route of elimination from the body. Mercapturic acid derivatives are found in the urine of rodents after administration but account for less than 20 percent of dose. Further information on the distribution of acrolein *in vivo*, dose-metabolism relationships, profiles of metabolism pathways across species, and relation of covalent binding to toxicity are needed.

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5. MAMMALIAN TOXICITY

5.1 ACUTE TOXICITY

The acute toxicity values for acrolein that were reported in the available literature are compiled in Table 5-1. Several of the values were based on range-finding studies that did not give complete experimental details as to age, strain, and number of animals. The early acute toxicity studies have been reviewed by Albin (1975).

5.1.1 Inhalation

Skog (1950) studied groups of eight rats exposed for 30 minutes to acrolein vapor at dose ranges between 100 and 200 mg/m^3 and observed the animals for up to 3 weeks. The LC_{50}, calculated by probit analysis, was 300 mg/m^3 (130 ppm), with all deaths occurring within 68 hours. Toxic signs observed in the animals were marked respiratory difficulties, eye irritation, lacrimation, and nasal discharge. Histopathologic changes in the lungs of the animals that died were hemorrhages and intraalveolar and perivascular edema.

Salem and Cullumbine (1960) studied the inhalation toxicity of acrolein as one substance of a series of saturated and unsaturated aldehydes. Groups of 50 mice, 20 guinea pigs, and 5 rabbits (strains unspecified) were exposed to either an aerosol or vapor of acrolein for up to 10 hours or until the animals died. Table 5-2 summarizes the results. The aerosol was produced by injecting air through an all-metal "Collision Spray" into 50 ml of acrolein in an all-glass container at room temperature; the mean particle size was 0.7 um diameter. Vapor was generated by air injection into acrolein warmed to 50 C. Aerosols and vapors were sampled and analyzed with hydroxylamine. The mice were found to be more susceptible to the acrolein exposure than the guinea pigs and rabbits; no differences were noted between administration by aerosol or vapor. Under the conditions of the study, acrolein was slightly more toxic than crotonaldehyde and considerably more toxic than acetaldehyde, propionaldehyde, and butyraldehyde; formaldehyde was not toxic. The animals exhibited eye irritation, labored breathing, signs of bronchial constriction,

TABLE 5-1. LETHAL DOSES AND LETHAL CONCENTRATIONS FOR ACROLEIN ADMINISTERED TO ANIMALS

Route	Species	Exposure time	Lethal dose	Reference
Inhalation	Mouse	1 min	approx LC_{50} 875 ppm (2013 mg/m^3)	Shell Chemical Corp. (1957)[a]
Inhalation	Mouse	10 min	approx LC_{50} 175 ppm (493 mg/m^3)	Shell Chemical Corp. (1957)[a]
Inhalation	Mouse	6 hr	LC_{50} 66 ppm (162 mg/m^3)	Phillipin et al. (1969)
Inhalation	Rat	10 min	LC_{50} 375 ppm (750 mg/m^3)	Catilina et al. (1966)
Inhalation	Rat	30 min	approx LC_{50} 150 ppm (345 mg/m^3)	Medical Dept., U.S. Army (1926)[a]
Inhalation	Rat	30 min	LC_{50} 131 ppm (300 mg/m^3)	Skog (1950)
Inhalation	Rat	4 hr	approx LC_{50} 8 ppm (18.3 mg/m^3)	Smyth, et at. (1951)
Inhalation	Cat	8 hr	approx LC_{50} 600 ppm (1570 mg/m^3)	ITIT (1975)
Inhalation	Hamster	4 hr	LC_{50} 25.4 ppm (58.4 mg/m^3)	Feron and Kruysse (1977)
Oral	Mouse	-[b]	approx LD_{50} 28 mg/kg	Shell Chemical Corp. (undated)[a]
Oral	Rat	-	approx LD_{50} 45 mg/kg	Smyth et al. (1951)
Oral	Rat	-	approx LD_{50} 42 mg/kg	Shell (undated)[a]
Subcutaneous	Mouse	-	LD_{50} 30 mg/kg	Skog (1950)
Subcutaneous	Rat	-	LD_{50} 50 mg/kg	Skog (1950)
Percutaneous	Rabbit	-	approx LD_{50} 200 mg/kg	Union Carbide (1956)[a]
Percutaneous	Rabbit	-	approx LD_{50} 562 mg/kg	Shell Chemical Corp. (1957)[a]
Intraperitoneal	Rat	-	Lethal dose 2.5 mg/kg	Carl et al. (1939)

[a] Albin (1975).

[b] Data not available.

TABLE 5-2. ACUTE INHALATION TOXICITY OF ACROLEIN

Species	Median lethal doses, x 10^5 mg x min/m^3	
	Vapor	Aerosol
Mouse	0.7	0.6
Guinea pig	1.3	1.1
Rabbit	1.4	1.2
	Mean Concentration, mg/m^3	
	5225	4624
	(2271 ppm)	(2010 ppm)

SOURCE: Salem and Cullumbine (1960).

and convulsions probably due to anoxia; death followed. On necropsy, the animals had expanded edematous and hemorrhagic lungs; those dying in the first 5 hours also had distended alveoli and ruptured alveolar septa.

The 4-hour LC_{50} in hamsters for inhaled acrolein was reported to be 25.4 ppm (58 mg/m^3) by Feron and Kruysse (1977).

An investigation of the interaction of acrolein and formaldehyde as sensory irritants was made by (Kane and Alarie, 1978). In this study, groups of four mice (Swiss-Webster strain, 20-30 g each) were exposed in inhalation chambers to varying concentrations of acrolein, formaldehyde alone, or the two in combination. Respiratory rates were measured as an index of sensory irritation; i.e., the greater the irritation, the more the animal suppresses its inhalations. Eleven 10-minute exposures were conducted in which the acrolein concentration ranged from 0.12 to 8.97 ppm and the formaldehyde concentration ranged from 0.33 to 9.73 ppm. The data from these exposures were used in a mathematical model developed to describe the interaction of agents acting at a single sensory receptor site. From the results, the investigators concluded that acrolein and formaldehyde act in competitive antagonism for the same receptor sites when causing sensory irritation.

5.1.2 Subcutaneous

The subcutaneous LD_{50}s of acrolein in mice and rats were 30 and 50 mg/kg, respectively. No toxic signs were noted except narcosis. All deaths occurred within 1 day. Histopathologic changes in the lungs were alveolar and perivascular edema without hemorrhage; these changes were less severe than those following inhalation exposure. Other histologic changes noted were hyperemia and fatty degeneration in the liver and inflammatory changes in the kidneys (Skog, 1950).

5.1.3 Oral

The oral LD_{50}s for mice and rats were 28 and 42-45 mg/kg, respectively (Table 5-1). Single doses of 10 mg/kg by gavage killed two of two rats, and doses of 5 mg/kg/day for 9 days were tolerated by six rats (Carl et al.,1939).

5.1.4 Intraperitoneal

Carl et al. (1939) suggested that acrolein was more toxic in rats by the intraperitoneal route than by oral gavage. Intraperitoneal injection of 2.5 mg/kg/day was lethal in all rats (number not specified) tested two days after administration of the test substance.

5.1.5 Dermal

When applied to the skin of rabbits, undiluted acrolein caused necrosis and a 1 percent aqueous solution caused burns.

5.1.6 Ocular

Instillation of a 1 percent solution into the eyes of rabbits caused severe (grade 10+) injury (Smyth et al., 1951).

5.2 SUBCHRONIC TOXICITY

Lyon et al. (1970) reported the results of four species of animals exposed to two different regimens: (1) acrolein vapor at levels of 0.7 or 3.7 ppm (1.6 and 8.5 mg/m^3) for 8 hours/day, 5 days/week for 6 consecutive weeks, and (2) acrolein vapor continuously for 90 days at levels of 0.21, 0.23, 1.0, and 1.8 ppm (0.48, 0.52, 2.3, and 4.1 mg/m^3). Test groups consisted of seven male and seven female NMRI:O Sprague-Dawley rats and Princeton or Hartley derived guinea pigs, nine male squirrel monkeys (Saim.ri sciurea), and two male beagle dogs. The data for the animals exposed continuously at 0.21 and 0.23 ppm were combined in reporting the study. Concentrations of acrolein in the exposure chamber were monitored several times daily. The initial weights of rats and guinea pigs in the various exposure groups varied widely; however, it appears that control groups of similar weights were used for comparison with each dosed group. Pre and postexposure body weights and hematology determinations were recorded, and immediately prior to study termination, blood urea nitrogen levels and serum alanine and aspartate aminotransferase activities were determined. Histopathologic examination of the heart, lungs, liver, spleen, and kidneys were made on all dogs and monkeys and on half of the rats and guinea pigs.

For the 0.7 ppm exposures, there were no toxic signs after repeated exposure. However, histologic examination of the lungs revealed mild chronic inflammatory changes and occasional mild emphysema. Inflammatory changes in the bronchi (mild infiltration of round cells) were more pronounced in monkeys and dogs than in other species.

During the exposure at 3.7 ppm, the dogs and monkeys showed eye irritation, excessive salivation, and labored breathing; these signs were less severe after the first week of exposure, although eye irritation persisted throughout the study (Lyon et al., 1970). Two monkeys died (days 6 and 9); both had pulmonary lesions. Rats and guinea pigs exposed repeatedly to 3.7 ppm acrolein vapor exhibited no pharmacotoxic reactions. Weight gains were signficantly lower in rats exposed at 3.7 ppm, but there were no effects on hematology or blood chemistry parameters in any species. Nonspecific inflammatory histologic changes were found in lung, liver, and kidney sections of all species. Squamous cell metaplasia and basal cell hyperplasia were noted in the tracheas of dogs and monkeys, and necrotizing bronchitis and squamous cell metaplasia of the lungs were found in seven of the nine monkeys exposed at 3.7 ppm acrolein.

In the studies using continuous exposure, no toxic effects were noted in any species at the 0.22 ppm exposure level. At exposure levels of 1.0 and 1.8 ppm, dogs and monkeys exhibited severe irritation as described for the repeated exposure study. Rats had decreased weight gains at both the 1.0 and 1.8 ppm exposure levels when compared to controls. Histologic changes after continuous exposure at 0.22 ppm acrolein paralleled those at the 0.7 ppm repeated exposure described above. At the 1.0 ppm level of continuous exposure, guinea pigs showed varying degrees of pulmonary inflammation and occasional foci of liver necrosis; three of nine rats examined histologically had foci of liver necrosis and occasional pulmonary hemorrhages; and the dogs had inflammation of the lungs, liver, and kidneys. At the highest level of exposure (1.8 ppm), all of the animals examined histologically had inflammatory changes in the lungs, liver, kidneys, brain, and heart; the histologic changes in the lungs and bronchi paralleled those found after repeated exposure at 3.7 ppm acrolein (Lyon et al., 1970).

Bouley et al. (1974, 1975) exposed 110 OFA male rats continuously to acrolein in air at 1.27 mg/m^3 (0.55 ppm) for up to 77 days; a control group of 110 rats was included in the study. Sneezing and nasal irritation were observed between days 7 and 21, and these signs disappeared thereafter. Mean body weights and food consumption were lower in the exposed group than in the control group throughout the study. After 60 days, the mean body weight of acrolein-exposed rats was approximately 80 percent of controls. The lung-to-body weight ratios were similar between exposed and control groups sacrificed at 15 and 32 days but were higher in the acroleinexposed group sacrificed after 77 days than in corresponding controls. Serum alkaline phosphatase was slightly but significantly decreased in exposed rats at day 15, but similar to control levels at 32 and 77 days. The absolute number of pulmonary macrophages was decreased at day 26 in exposed rats when compared to controls. There was an accompanying increased susceptibility of acrolein-exposed animals to experimental aerosol infection with Salmonella enteritis at day 18, but not day 24; the death rate in rats exposed to acrolein and infected by airborne Salmonella at day 18 was 15/16 compared to 8/15 for controls. After 63 days, similar death rates due to experimental Salmonella infection were found in both exposed and control groups (10/10).

Feron et al. (1978) studied the effects of repeated exposure to acrolein vapor in Syrian golden hamsters, SPF Wistar rats, and Dutch rabbits. Groups of 20 hamsters, 12 rats, and 4 rabbits of each sex were exposed to acrolein vapor at 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 11.3 mg/m^3) for 6 hours/day, 5 days/week for 13 weeks. Body weights and food consumption were measured weekly. Hematology, clinical chemistry, and urinalysis determinations were performed at 12 weeks. At study termination, necropsies were performed, organ weights measured, and histopathology performed on all control and high dose animals. No toxic signs were noted in any species at the 0.4 ppm exposure level. At the 1.4 ppm level, the rabbits exhibited some sneezing and the rats and hamsters appeared narcotized. Increased salivation and nasal secretion and eye irritation were noted in all animals exposed to acrolein at the 4.9 ppm level. At the highest dose level, deaths were reported in 3/12 male and 3/12 female rats. One hamster in the high dose (4.9 ppm) group was sacrificed moribund at week 12 with renal failure. There was a decreased weight gain in rats and rabbits exposed at both 1.4 and 4.9 ppm and in hamsters exposed at 1.4 ppm. There were no effects of exposure on hematologic or clinical chemistry parameters. There were increases in organ-to-body weight ratios in animals exposed at the highest level. At 4.9 ppm, there were increases in relative weights of lungs in hamsters, rats, and rabbits; of the heart and kidneys in hamsters and rats; and of adrenals in rats as compared with controls. At the highest exposure level, marked histologic changes were seen in the epithelium of the nasal cavity of all species; there was necrosis and neutrophil infiltration of the mucosa. At an exposure level of 1.4 ppm acrolein, squamous metaplasia and neutrophil infiltration of the nasal mucosa of rats was observed although a similar effect in hamsters was not found. Hyperplasia in the trachea was seen in all species at the highest exposure level; hyperplastic effects were more severe in rats than in hamsters or rabbits. Histologic changes in the lungs and bronchi, hemorrhage, and perivascular and aleveolar edema were found in rats and rabbits exposed at 4.9 ppm, whereas no effects were noted in hamsters. On the basis of this study, the descending order of species sensitivity was rats, rabbits, hamsters.

5.3 CHRONIC TOXICITY

Feron and Kruysse (1977) performed a study to determine if acrolein acted as a cocarcinogen in respiratory carcinogenesis induced by benzo(a)pyrene or diethylnitrosamine. They exposed a control group of 18 male and 18 female Syrian golden hamsters at 4 ppm (9.2 mg/m^3) acrolein for 7 hours/day, 5 days/week for 52 weeks and observed some of the animals until week 81. Eye irritation, excessive salivation, and nasal discharge were noted during the first week of the study; however, the animals adapted and the signs were no longer observed. The mean body weights of both exposed males and females were lower than those of the controls during the exposure period. The differences decreased in the postexposure period. Lung weights were increased in exposed animals compared to controls, and there was a moderate degree of inflammation and epithelial metaplasia in the nasal cavity; these changes persisted through the postexposure period (weeks (56-81). There were no other histologic changes in the respiratory system related to acrolein exposure. Acrolein did not augment the effects of benzo(a)pyrene or diethylnitrosamine.

5.4 EFFECTS ON THE LIVER, KIDNEYS, AND LUNGS

5.4.1 Liver

Murphy et al. (1964) studied the effects of acrolein on liver enzymes after it was administered to rats by intraperitoneal injection or when the rats were exposed to acrolein vapor. Groups of four adult male Sprague-Dawley rats were injected intraperitoneally with acrolein; the average liver alkaline phosphatase (AP) levels 24 hours later were 84, 113, 170, and 368 percent of controls at dose levels of 0.38, 0.75, 1.5, and 3.0 mg/kg, respectively. Table 5-3 shows the effect of acrolein on liver-to-body weight ratios and liver AP activity. In a separate experiment, continuous exposure at 4 ppm acrolein for 4, 8, and 20 hours resulted in liver AP activities that were 135, 222, and 233 percent of their respective controls. Subsequent studies indicated that the effect of acrolein on liver AP was secondary to stimulation of hypersecretion of glucocorticoids by the adrenals. The effect on AP activity elicited by acrolein was prevented by adrenalectomy and hypophysectomy. Tyrosine amino Tyrosine amino transferase activity was also increased in the livers of acrolein-treated rats (Murphy, 1965; Murphy and Porter, 1966). Szot and Murphy (1970, 1971) studied the effect of single sublethal doses (intraperitoneal) of acrolein on plasma and adrenal corticosterone in male Holtzman rats. Doses between 0.1 and 6.0 mg/ml acrolein were given to rats, and corticosteriod levels were measured after 1 hour. Levels of adrenal and plasma corticosterone increased 300500 percent of control levels at increasing dose levels of acrolein. Dexamethazone partially reversed the effect of a low dose of acrolein (0.10 or 0.25 mg/kg) but not of high doses (1.50 or 6.0 mg/kg).

TABLE 5-3. EFFECT OF ACROLEIN ON LIVER ALKALINE PHOSPHATES AND RELATIVE LIVER WEIGHT

Concentration, ppm	No. of animals	Time, hr	Mean liver AP, % of control	Increased liver-to-body weight ratio
4.1	6	20, continuous	233	+
2.1	12	41, continuous	148	+
1.0	12	81, continuous	104	-
4.0	6	4/day x 5 days	100	-
3.9	6	4/day x 9 days	71	-

Source: Murphy et al. (1964).

Butterworth et al. (1978) found periportal necrosis in weanling male Wistar-derived rats 24 hours after intravenous infusion of acrolein (0.85 or 1.70 mg/kg) into the mesenteric vein.

5.4.2 Kidneys

Mild effects on the kidneys were noted in section 6.1.2 (Skog, 1950).

5.4.3 Lungs

Murphy et al. (1963) measured pulmonary function in guinea pigs exposed to nonlethal doses of acrolein. Random-bred male guinea pigs were exposed at levels of acrolein vapor between 0.4 and 1.0 ppm via facemask, under dynamic flow conditions, for 2 hours. Respiratory rate, tidal volume, and flow resistance were measured with a plethysmograph. There was a dose-related increase in total respiratory flow resistance accompanied by a decreased respiratory rate and increased tidal volume. The changes were reversible. Acrolein appeared to mediate its effects by bronchoconstriction, since anticholinergic drugs known to relieve bronchoconstriction partially or completely reversed the effects. The levels of acrolein used were near or below the levels of human sensory detection.

Watanabe and Aviado (1974) exposed male Swiss mice to acrolein for 5 weeks at a level of 0.1 mg/l (0.1 ppm) or for 5 minutes at levels of 0.3 or 0.6 mg/l (0.3 or 0.6 ppm). The effects were a decreased pulmonary compliance, decreased tidal volume, and decreased respiratory rate.

Kane and Alarie (1977) studied the effect of exposure to acrolein on the respiratory rate of Swiss Webster mice. Groups of four mice were exposed at varying levels of acrolein vapor in a glass chamber in which the heads only were exposed to the atmosphere and the bodies enclosed in a plethysmograph that was connected to external recorders. There was a dose-response decrease in respiratory rate during a 10-minute exposure. The RD_{50} (50 percent decrease in respiratory rate) was 1.7 ppm (3.9 mg/m^3). This was calculated by linear regression of a graph of the logarithm of concentration vs percent decrease in respiratory rate at levels of acrolein between 0.2 and 10 ppm. Acrolein was about twice as potent as formaldehyde, which had an RD_{50} of 3.1 ppm. By exposing the mice while they were breathing through a tracheal cannula, it was shown that the primary effect is on the upper respiratory tract. When the mice were exposed at 0.4 or 1.7 ppm acrolein vapor for 3 hours/day for 4 days, and then tested on the 5th day for an acute RD_{50}, Kane and Alarie found that the response was greater than expected (hypersensitivity). However, when the mice were exposed on 3 consecutive days at one-tenth the RD_{50} (0.17 ppm) and tested on the 4th day, there was a development of tolerance and a decrease in the expected response.

The mucoid layer of the upper respiratory tract is a complex system of mucus and cilia that protects the organism by clearing the upper respiratory tract of foreign material (Carson et al., 1966). Kensler and Battista (1966) found that acrolein was one of the components of tobacco smoke that inhibited ciliary transport activity in isolated tracheal preparations from chickens. In this study, there were no quantitative data on the levels of acrolein causing an effect. In another study, Battista and Kensler (1970) reported the effects of cigarette smoke and acrolein on ciliary transport activity in the tracheas of chickens *in vivo*. Smoke was passed through absolute filters to remove acrolein; measured amounts of acrolein were then added to the smoke, and the smoke was administered to the chickens. The ED_{50} for acrolein based on eight puffs (e.g., one cigarette equivalent) was 3040 ug acrolein/40 ml puff. A 40 ml puff of unfiltered cigarette smoke contains between 8 and 20 ug of acrolein. Dalhamn and Rosengren (1970) confirmed the effect of acrolein on cilia of isolated trachea of cats. Ciliostasis was reported at aerosol levels of 50100 mg/m^3 (2243 ppm) acrolein.

Astry and Jakab (1983) exposed female Swiss mice to acrolein vapor for 8 hours at levels of 3 and 6 ppm (6.9 and 13.8 mg/m^3); a control group was also included in the study. Following the exposure to acrolein, the mice received an aerosol of ^{32}Plabeled *Staphylococcus aureus*. Groups of animals were sacrificed immediately after the bacterial challenge and after 8 hours. The lungs were removed and homogenized. Viable bacterial counts and ^{32}P levels were determined at both time intervals, and the percent of initial viable staphylococci remaining in the lungs was determined. In control animals there was 95 percent intrapulmonary killing of *S. aureus* after 8 hours. Approximately 50 percent of the initial viable bacteria remained in the lungs after exposure at 3 ppm; after exposure at 6 ppm, the pulmonary antibacterial defenses were totally repressed, allowing growth of *S. aureus* to 150 percent of the initial challenge.

The lowered defense against infection by bacteria noted in the above study and in the study of Bouley (1974, reviewed in section 6.2) correlate with the results of two other studies. In the study by Rylander (1973), the author deduced that acrolein was one of the components of cigarette smoke that caused a decrease in the number of macrophages in the free lung cells of guinea pigs inhaling cigarette smoke. In the study by Kilburne and McKenzie (1970), the authors found that acrolein in combination with carbon caused recruitment of "scavenging" cells to the respiratory system and was cytotoxic to these cells whose normal homeostatic function is host protection against microbial infection. Groups of 614 Syrian golden hamsters, weighing approximately 100 g each, were exposed to acrolein aerosol (6 ppm) and carbon (1.4 um particle size diameter, 6 ppm) for 6-96 hours; less than 10 percent of that to which the hamsters were exposed was inhaled. After various periods of exposure, groups of animals were sacrificed and the number of leukocytes "recruited" to airway cells of the trachea and lungs was measured by electron microscopic examination of tissue sections. Polymorphonuclear leukocytes (PMNs) were recruited to both tracheal and bronchiolar cells with a peak of accumulation occurring at 24-48 hours and then subsiding. Neither carbon nor acrolein alone caused the effect. Absorption of carbon was necessary for the chemotactic effect of acrolein on the respiratory system, but it was shown that acrolein by itself can cause a cytotoxic effect on airway cells. Cytotoxicity of acrolein to cultured mammalian cells is discussed in section 6.5.2.6.5

5.5 OTHER STUDIES

5.5.1 Cardiovascular

Egle and Hudgins (1974) studied the sympathomimetic and cardiovascular effects of injected and inhaled acrolein on anesthetized male Wistar rats. A low concentration of acrolein (9.25 mg/kg) was administered by intravenous injection, and a rise in blood pressure (pressor effect) was noted in most rats, which began within 5 seconds, reached a peak within 30 seconds, and subsided in approximately 1 minute. Higher loses of acrolein depressed blood pressure in most animals. Groups of 6-11 animals were exposed 16-28 times each to acrolein levels ranging from 0.01 to 5.00 ppm. Inhalation of levels of 0.01 ppm acrolein caused a pressor effect; higher doses frequently caused a cardio-inhibitory effect. It was suggested that the pressor effect was mediated by release of neurotransmitters from sympathetic nerve endings as well as by an action on the adrenal medulla.

Green and Egle (1983) studied the effect of acrolein on blood pressure in an inbred hypertensive strain of Wistar rats and compared the effects with the same strain of rats receiving the antihypertensive agent guanethidine. Doses of 0.05 and 0.1 mg/kg acrolein administered intravenously to anesthetized rats caused a pressor effect, whereas decreased blood pressure was noted at doses of 0.3, 0.4, and 0.5 mg/kg acrolein. The responses were not altered by guanethidine. Uniform responses were not observed in all animals in each group.

5.5.2 In Vitro Cytotoxicity

Several studies have investigated the *in vitro* cytotoxicity of acrolein in cultured mammalian cells. Results of the studies are summarized in Table 5-4. Holmberg and Malmfors (1974) found that acrolein was substantially more cytotoxic in Ehrlich-Landschultz ascitis cells than was formaldehyde, and that both aldehydes were among the most toxic organic solvents in the series studied. The cytotoxic effects of acrolein may be due to effects on nucleic acid and protein synthesis. Leuchtenberger et al. (1968) studied the uptake and incorporation of ^{3}H-uridine in primary mouse kidney cultures. The cells were exposed to 5-second pulses of acrolein vapor (approximately 5 ug/pulse) every 15 seconds for a total exposure of approximately 400 ug. There was a progressive dose-related decrease of uptake of ^{3}H-uridine, an inhibition of RNA synthesis, and pycnosis of the cell nuclei. Moule and Frayssinet (1971) and Munsch et al. (1973) compared the effects of acrolein on DNA and RNA polymerases of rat liver and *Escherichia coli*. It was concluded (Munsch et al., 1974) that the effect of acrolein on DNA synthesis was mediated by the reaction of acrolein with the sulfhydryl group of the polymerase. Rat liver DNA polymerase has a functional sulfhydryl group, whereas the *E. coli* polymerase does not, and rat liver DNA polymerase bound about 25 times as much ^{3}H-acrolein as did the *E. coli* enzyme (Munsch et al., 1973).

5.6 EFFECTS ON HUMANS

Acrolein vapor has been found to be an extremely potent eye and respiratory tract irritant in humans. Table 5-5 summarizes ocular responses to acrolein in humans, and Table 5-6 summarizes threshold levels for responses. Sim and Pattle (1957) studied experimental human exposure to several aldehydes. Extreme irritation was noted in humans exposed to acrolein at levels of 0.80 ppm (1.8 mg/m^3) for 10 minutes or 1.22 ppm (2.8 mg/m^3) for 5 minutes. Lacrimation occurred after 20 seconds at 0.8 ppm and after 5 seconds at 1.22 ppm. For

TABLE 5-4. *IN VITRO* CYTOTOXICITY OF ACROLEIN IN MAMMALIAN CELL CULTURES

Cell culture	Exposure conditions	Effects	Reference
Ehrlich-Landshultz ascites tumor cells	1-100 ppm as a vapor, up to 5 hr	Partial cell death at 5 ppm; total cell death at 100 ppm	Holmberg and Malmfors (1974)
C 1300 mouse neuroblastoma cells	Concentrations not given, 24 hr	Decreased viability at 30 μM; increased sloughed cells at 60 μM	Koerker et al. (1976)
Chinese hamster ovary (CHO) cells	10-100 μM, 5 hr	Decreased mitosis at 10 μM; chromosomal tangling at 100 μM	Au et al. (1980)
Walker ascites tumor cells	Concentrations not given, 1 hr	50% inhibition of division at 1.0 μg/ml (180 μM)	Phillips (1974)
Ascites sarcoma BP8 cells	10-100 μM, 48 hr	20% inhibition of growth at 10 μM; 91% at 100 μM	Pilotti et al. (1975)
HeLa 53 cells	Concentrations not given, 2.5 hr	Too toxic to measure unscheduled DNA synthesis	Schiffman et al. (1983)
Rat thymic lymphocytes	10-40 μM, 72 hr	Decreased viability at 30 μM	Hussain et al. (1983)

TABLE 5-5. OCULAR RESPONSE TO AIRBORNE ACROLEIN

Concentration, ppm	Duration of exposure	Effect	Reference
0.8	10 min	Extremely irritating; only just tolerable	Sim and Pattle (1957)
1-2	5 min	87% of test panel reported irritation	Kane and Alarie (1977)
1	5 min	82% of test panel reported irritation	Kane and Alarie (1977)
0.5	5 min	35% of test panel reported irritation	Kane and Alarie (1977)
0.5	5 min	19% of test panel reported irritation	Kane and Alarie (1977)
0.5	12 min	91% of test panel reported irritation	Kane and Alarie (1977)
1.8	1 min	Slight irritation	Kane and Alarie (1977)
1.8	2 min	Distinct irritation	Kane and Alarie (1977)
1.8	4 min	Profuse lacrimation; practically intolerable	Kane and Alarie (1977)
5.5	5 sec	Moderate irritation	Kane and Alarie (1977)
	20 sec	Painful irritation	Kane and Alarie (1977)
	60 sec	Marked lacrimation; practically intolerable	Kane and Alarie (1977)
21.8	--[a]	Intolerable	Kane and Alarie (1977)
30.25	5 min	Moderate irritation	Kane and Alarie (1977)
4	4 min	Severe irritation	Schuck and Renzetti (1960)
0.06	--	Irritation 0.471 on scale of 0-2	Darley et al. (1960)
1.3-1.6	--	Irritation 1.182 on scale of 0-2	Kane and Alarie (1977)
2.0-2.3	--	Irritation 1.476 on scale of 0-2	Kane and Alarie (1977)

[a] Data not available.

TABLE 5-6. THRESHOLDS OF RESPONSE AFTER EXPOSURE TO ACROLEIN

Concentration, ppm	Response
0.2	Eye irritation threshold
0.33-0.40	Odor threshold
0.40-1.0	Prolonged deep respiration
0.62	Respiratory response threshold
1.0	Immediately detectable
5.5	Intense irritation

SOURCE: National Research Council (1981).

comparison, crotonaldehyde was highly irritating (4.1 ppm for 15 minutes); acetaldehyde was slightly irritating; and propionaldehyde, butyraldehyde, and isobutyraldehyde were nonirritating. The threshold level for eye irritation due to acrolein exposure is 0.2 ppm (0.46 mg/m^3) (Table 5-6); formaldehyde, by comparison, is about 2.5 times less potent (Altschuller, 1978).

Acrolein has been identified as one of the main components of smog that acts as an eye irritant (Renzetti and Bryan, 1961; Altschuller, 1978). Acrolein is a component of diesel exhaust and can be produced experimentally by photooxidation of hydrocarbons in the presence of nitrous oxides (Schuck and Renzetti, 1960). It is also a component of cigarette smoke and is a major contributor to the irritant property of cigarette smoke (Weber-Tschopp et al., 1976).

Two cases of occupational exposure to acrolein (one fatal) have been reported by NIOSH (National Research Council, 1981). It has been speculated that the greatest occupational danger of acrolein exposure is associated with the welding of fat and oil cauldrons. Because of the acutely irritating properties of acrolein, human exposure is likely to be limited and thus observed toxic effects rare (National Research Council, 1981).

A case of accidental exposure to acrolein was reported by Champeix et al. (1966) in which a chemical worker was accidentally sprayed in the face with acrolein. He had immediate burning of the eyes and face, and within 20 hours was hospitalized with thoracic constriction, cough, frothy sputum, and cyanosis. Other signs included swollen eyelids and a rapid respiratory rate. After 9 days of hospitalization and treatment, the patient was released with a moderate cough. Eighteen months later he developed a chronic bronchitis and emphysema possibly related to the acrolein exposure and injury to the respiratory system.

Gosselin et al. (1979) reported a case of two boys (ages 2 and 4.5 years) who accidentally inhaled smoke from an overheated fryer for 2 hours. The smoke contained acrolein. The younger boy was found dead, and the other was hospitalized with respiratory difficulties and given oxygen therapy. Death due to asphyxiation occurred within 24 hours. On autopsy, massive cellular destruction in the trachea and bronchi and pulmonary infarcts were found.

Bauer et al. (1977) reported a case of acrolein poisoning caused by inhaling vapors from vegetable cooking oil that had been overheating for 6 hours in a small kitchen. The subject experienced pulmonary changes that were life threatening. It was concluded by the authors that acrolein was the major toxic component of the inhaled vapor.

In its liquid form, acrolein has caused severe skin irritation in humans. Dermal application of a 1 percent solution produced a positive patch test (National Research Council, 1981).

Some information on the metabolism of acrolein in humans was produced by studies of patients receiving cyclophosphamide. This drug, which is used in cancer chemotherapy, is generally believed to be metabolized to phosphoramide mustard and acrolein (Alacron, 1976; Low et al., 1983). Two studies have identified S-hydroxypropylmercapturic acid (MCA) in the urine of patients given cyclophosphamide, suggesting that acrolein may in part be converted to the mercapturate as had been found in rats. In one study (Kaye and Young, 1974), the urine of two patients receiving cyclophosphamide orally (50 mg twice daily and 50 mg three times daily, respectively) was collected over 24 hours, and MCA was identified in both samples by paper chromatography. In the second study (Alacron, 1976), cyclophosphamide (1 g intravenously, equivalent to 10-18 mg/kg) was given to five patients who were receiving multiple drugs for unspecified illnesses, and their urine was collected over 6 hours. The amounts of urinary MCA found varied widely from 6.4 to 50.0 umol; no correlation between dose and urinary MCA was evident.

5.7 SUMMARY

Toxicity studies with acrolein indicate that it is highly toxic by the inhalation and oral routes of exposure, moderately toxic through skin absorption, and is a strong skin and eye irritant. The descending order of sensitivity in acute inhalation exposure to acrolein is mouse, rat, guinea pig, cat. The approximate LC_{50} for a 10minute exposure in mice was 175 ppm (493 mg/m^3) and in rats 375 (750 mg/m^3); however, concentrations on the order of 12 ppm acrolein vapor were irritating to the eyes and nose of experimental animals after an exposure period of 12 minutes. Mice were more sensitive than rats when given acrolein orally, approximate LD_{50}s being 28 and 45 mg/kg, respectively; subcutaneous LD_{50}s were in a similar range. One percent aqueous solutions of acrolein were extremely irritating when applied to the skin or instilled in the eyes of rabbits. Acute inhalation exposure to acrolein in animals caused bronchiolar constriction resulting in anoxia and death. Histologic changes in animals that died after exposure were found in the nasal passages, bronchi, trachea, and lungs.

Subchronic inhalation studies of acrolein have been conducted using both repeated exposure for 6 hours/day, 5 days/week for 6 weeks or using continuous exposure for 6 weeks with monkeys, dogs, rabbits, guinea pigs, hamsters, and rats. A no observed effect level (NOEL) in all species was 0.4 ppm (0.92 mg/m^3). Repeated exposure at 0.7 ppm acrolein for 6 weeks caused no toxic effects in monkeys, dogs, guinea pigs, or rats, but histologic examination revealed mild inflammatory changes in the bronchi and lungs that were more severe in monkeys and dogs than in rats and guinea pigs. Levels between 3.7 and 4.9 ppm (8.5 and 11.3 mg/m^3) caused severe toxic signs and some degree of histologic change in tissues of the respiratory tract in all species tested. Repeated exposure at 3.7 ppm (8.5 mg/m^3) acrolein caused toxic effects (irritation, lacrimation, and nasal discharge) in dogs and monkeys but not in rats and guinea pigs.

In a study comparing species sensitivity to inhalation exposure to acrolein, it was found that the descending order of sensitivity was rats, rabbits, hamsters. After repeated exposure to acrolein at 4.9 ppm (11.3 mg/m^3), the nasal cavities, trachea, and bronchi showed histologic aberrations consisting of destruction, hyperplasia, and metaplasia of the epithelia of the airways and neutrophil infiltration of the mucosa; similar changes were seen in hamsters exposed to acrolein at 4 ppm (9.2 mg/m^3) for 7 hours/day, 5 days a week for 52 weeks.

Continuous exposure at 0.22 ppm (0.5 mg/m^3) and 1.0 ppm (2.3 mg/m^3) acrolein produced similar results to repeated exposures at 0.4 ppm and 4.9 ppm, respectively. Histologic changes in the respiratory tract were generally more severe in monkeys and dogs than in rats or guinea pigs.

Reduced pulmonary compliance and tidal volume and a decrease in respiratory rate were reported after both acute and long-term exposure of mice to levels of acrolein below those that cause toxic effects. Inhalation of levels of acrolein equivalent to those found in cigarette smoke reportedly caused an inhibition of ciliary transport. This effect correlates with decreased pulmonary antibacterial defenses in experimental animals exposed to acrolein.

Although the primary target site of acrolein exposure appears to be the respiratory system, effects have also been noted on the liver, kidneys, and cardiovascular system. An increase in liver enzymes in rats receiving acrolein by the intraperitoneal route or by inhalation may be mediated by hypersecretion of glucocorticoids by the adrenals. Effects on the kidneys are not well defined. Cardiovascular effects, primarily a transient increase in blood pressure, may be sympathomimetic in nature.

In vitro studies indicate that the mechanism of the toxic effects of acrolein may be a binding of essential thiol groups in proteins that affects macromolecular biosynthesis and causes cell death.

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6. MUTAGENICITY

6.1 GENE MUTATIONS

6.1.1 Salmonella

There are more than 10 articles in the published literature which report on the testing of acrolein in one or more strains of Salmonella. Those that were abstracts or publications reporting the results of a large number of chemicals in which only statements of positive/negative were reported are not included in this evaluation.

The most extensive testing of acrolein was reported by Lijinsky and Andrews (1980) who employed the plate incorporation test using strains TA1535, TA1537, TA1538, TA98 and TA100 without activation and with added rat or hamster liver S-9 from animals induced with Aroclor 1254. Acrolein was initially tested over a broad concentration range both in dimethyl sulfoxide (DMSO) and distilled water. Acrolein was highly toxic and positive mutagenic results were seen only in strain TA98 in the absence of S-9 when acrolein was dissolved in distilled water. Representative data from one of the replicate experiments were reported in which acrolein was tested from 0.001 to 0.1 µl/plate in increments of 0.01 above .01 µl/plate. The response increased with dose up to 0.03 µl/plate at which the response was approximately 2.5 times the control level. At higher concentration the number of revertants decreased and was just twofold above controls at 0.1 µl/plate.

Lutz et al. (1982) used TA100 to test acrolein and several other unsaturated aldehydes in a 90 minute preincubation test. Acrolein was tested at 0.0125, 0.0375, 0.075, 0.1125 and 0.15 micromoles/plate both without and with rat liver S-9 activation. A positive dose related response was reported only without S-9 and in least two independent experiments. The data were presented only in graphical form and interpolation from the graph indicates approximately 430 revertants/plate at the highest concentration compared to approximately 130 revertants/plate for controls. An aliquot of the preincubation suspension was also diluted and plated on complete medium for determination of survival. While it is not possible to interpolate with any accuracy from the toxicity graph it appears that survival was very low at the high concentration.

Hales (1982) tested acrolein at one log concentration intervals from 0.001 to 10 µg/plate (10 µg/plate = 0.178 micromoles/plate) only in TA1535. Acrolein was extremely toxic both with and without S-9 activation and was reported not mutagenic. Because only log intervals were tested and because only TA1535 was used, these results cannot be compared with other reports.

The National Toxicology Program (Haworth et al. 1983) used TA1535, TA1537, TA98 and TA100 in a preincubation assay to test acrolein. Metabolic activation was provided by both rat and hamster liver S-9. Concentrations of acrolein tested included half-log increments from 0.03 to 100 µg/plate and several other concentrations between 10 and 100 µg/plate. Severe toxicity was observed in all strains at less than 10 µg/plate without activation, with no evidence of mutagenicity. Higher concentrations were tolerated with both rat and hamster S-9 but no clear mutagenic response was observed. They did observe a dose-related trend in TA100 with rat S-9 with a maximum response about 1.8 times concurrent controls at 50 µg/plate. A repeat of the TA100 assay was later conducted (Haworth, personal communication) at concentrations of 0, 1, 3.3, 10, 33 and 100 µg/plate. Again negative responses were seen without S-9 and with hamster S-9. With rat S-9 the number of revertants was not different from controls up to 10 µg/plate, was 2 times controls at 33 µg/plate, and total toxicity prevailed at 100 µg/plate.

Marnett et al. (1985) tested acrolein using a new Salmonella strain TA104. This is a base pair substitution strain which carries a non-sense mutation at the reversion site and, along with TA102, was designed to detect peroxides and other oxidants. Concentrations of acrolein were not listed but interpolation from the graphical presentation of the data indicates that they tested at least 0.1, 0.2 and 0.375 micromoles/plate. While they stated that 0.9 micromoles/plate was the maximum non-toxic concentration, no mutagenicity data were reported for this concentration. A dose-related response was observed with a doubling of control valves occurring at about 0.375 micromoles/plate.

It is clear that the results of these studies, as well as those not presented due to their sparse reporting, do not present a consistent interpretation of the mutagenic activity of acrolein in Salmonella. To a large extent this appears to be due to the extreme toxicity of acrolein to bacteria and therefore the variations in concentrations used and minor differences in protocols are especially critical. It is further noted that the maximum mutagenic response obtainable before cell toxicity predominates was approximately three-fold over the background rate. This point is raised here because there is a tendency to compare mutagenic potencies on the basis of induced mutants per unit of concentration (often revertants per nanomole for Samlonella), as was done in some of the articles cited above. The value of this comparison becomes questionable when chemicals with widely varying toxicity are compared on this basis.

6.1.2 E. coli

Ellenberger and Mohn (1977) used strain K12/343/113, which is capable of detecting both forward and reverse mutations at several loci, to evaluate the mutagenicity of acrolein. Cells were incubated with nine concentrations of acrolein ranging from 0.05 to 0.70 mM for 3 hr and aliquots were plated on complete and selective media for survival and mutation detection, respectively. Interpolation from a toxicity curve reported shows a dramatic increase in toxicity beginning at 0.45 mM (50 percent survival). At 0.7mM survival was less than 0.5 percent of controls. The actual mutagenicity data were not reported, but the authors stated that in several experiments acrolein did not exhibit mutagenic activity.

Hemminki et al. (1980) reported acrolein as a weak mutagen in strain WP2 uvrA (trp-) of E coli. Acrolein was one of many chemicals reported and the concentrations were reported only as being in the range of 20 to 10,000 micromolar for all chemicals. Also, the results were reported only as induced mutant frequency per micromole without reporting the control frequency, or the level of toxicity. Therefore, it is not possible to verify the conclusion reported.

6.1.3 Yeast

Izard (1973) reported both positive and negative responses in yeast when different endpoints were measured. The positive was observed in the induction of "petite" mutants; a doubling of the control incidence at 50 percent survival after treatment with acrolein at 320 mg/l (5.7 mM). Since only the percent response was reported, it is not clear that a two-fold increase is actually significantly different from controls. Acrolein, tested at concentrations ranging from 6.25 mg/l to 100 g/l, did not induce an increase in either of two yeast strains capable of detecting reverse mutations at the methionine locus. The author did not remark on toxicity in this experiment, but in the light of the toxicity encountered in the strain used to measure "petite" mutants, a concentration of 100 g/l seems implausible (a typographical error in the report is more likely). Neither of the strains used by Izard have been extensively used, hence it is not possible to evaluate this study in the context of current work in yeast.

6.1.4 Drosophila

The first report on the mutagenicity of acrolein was that of Rapaport (1948). Drosophila larvae were allowed to feed on nutrient medium containing acrolein or other unsaturated aldeheydes. The incidence of sex-linked recessive lethals in the survivors of acrolein treatment was 2.23 percent as compared to 0.19 percent in controls. The concentration of acrolein used was not reported but the level used was reported to cause death in greater than 75 percent of the larvae treated. There is also no mention of whether mutational clusters were considered; this is especially important since larval treatment involves treatment of only primordial germ cells and clusters are almost certain to appear. More recently, Zimmering et al. (1985), under contract with the National Toxicology Program, tested acrolein in the more conventional protocol for sex-linked recessive lethals by treating adult males. Acrolein was fed at 3,000 ppm in 5 percent sucrose or injected at 200 ppm in saline with appropriate vehicle controls and the incidence of recessive lethals was essentially identical to controls (0.05-0.07 percent).

6.2 CHROMOSOMAL EFFECTS

In an investigation of cyclophosphamide and its metabolites Au et al. (1980) tested acrolein for chromosome breaks and sister chromatid exchange (SCE) in Chinese hamster ovary cells (CHO). For the analysis of chromosome breaks, cells were exposed for 5 hr to acrolein at 0, 10, 40 and 100 μM both without and with rat liver S-9 activation. Extreme toxicity at all levels without S-9 precluded a measure of breaks and with S-9 they observed a doubling in the incidence of breaks at 40 μM acrolein, but the high variability and the fact that the incidence at 10 μM was actually lower than controls led the authors to conclude that the response was not a true indication of a mutagenic effects of acrolein. For SCE analysis cells were exposed for 1 hr at 0, 5, 10, 20 and 40 μM acrolein, washed and incubated with Brd U for 24 hr for the visualization of SCE. A dose-related increase in SCE was recorded without S-9; 10 μM was an approximate doubling of controls and 40 μM was toxic to the cells. Recently, the National Toxicology Program also employed these same assays with different treatment times and concentrations of acrolein (Zeiger, personal communication). Acrolein was tested at 0, 0.1, 0.3 and 1.0 μg/ml (approximately 0, 1.8, 5.4 and 18 μM, respectively) and CHO cells were exposed for 48 hrs without activation and 2 hr with rat liver S-9. For total chromosome aberrations without S-9 the response at the highest dose was marginally significantly higher than controls but there was not a dose-related increase. For SCE without S-9 a dose-related positive response was observed. Both endpoints were negative in the presence of S-9. Given the differences in protocols and concentrations, these results of two studies are quite similar.

The only in vivo mammalian study on acrolein was a dominant lethal assay in ICR male mice reported by Epstein et al. (1972). Males 8-10 weeks old received single IP injections of 1.5 or 2.2 mg/kg acrolein and were mated with 3 untreated females per week for 8 weeks. No data were reported but the authors stated that the incidence of early fetal deaths and preimplantation losses were within control limits.

6.3 SUMMARY OF MUTAGENIC EFFECTS

The majority of the mutagenicity tests on acrolein have employed bacterial systems and both positive and negative results have been reported. Differences in bacterial strains tested, protocols used and the differences in concentrations tested preclude a reconciliation of the apparently conflicting results. All reports do indicate that acrolein is extremely toxic with significant toxicity noted between 0.1 and 1 μmoles/plate and complete toxicity at less than 5 μmoles/plate in Salmonella.

In eukaryotes, acrolein did not induce gene mutations in methionine requiring strains of yeast, but did induce mitochondrial "petite" mutations in another yeast strain. Acrolein induced sex-linked recessive lethals in Drosophila when larvae were treated but not when adult males were treated in the currently conventional procedure.

Acrolein did induce sister chromatid exchange in mammalian cells in vitro but only in the absence of exogenous S-9 activation. Extreme toxicity precluded detection of chromosome aberrations in these cells. Finally, acrolein was reported negative in a mouse dominant lethal test in which males received a single IP injection of 1.5 or 2.2 mg/kg.

Applying the weight-of-evidence scheme of the proposed Guidelines for Mutagenicity Risk Assessment (Environmental Protection Agency, 1984) to the acrolein data, results in the classification of "Inadequate evidence bearing on either mutagenicity or chemical interactions with mammalian germ cells." The basis for this conclusion is the absence of data for mammalian gene mutations and in vivo mammalian cytogenetics data (other than the very limited dominant lethal test), and lack of data on mammalian germ cell interaction.

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7. CARCINOGENICITY

The bioassay literature on acrolein carcinogenicity is limited to skin painting, and subcutaneous injection studies with mice and an inhalation study using hamsters, all with essentially negative results. these studies, however, do not provide sufficient data for a definitive evaluation of the cancer endpoint. Bioassay evaluations of other substances with related chemical structures including an acrolein metabolite provides an insight into the potential for acrolein to elicit a carcinogenic response. Glycidaldehyde, which is one of the isolated metabolites of acrolein in rodents (see the metabolism and pharmacokinetics section) has also been tested by skin application or subcutaneous injection. Results of these studies indicate a statistically significant increase of local site tumors.

7.1 ANIMAL STUDIES

7.1.1 Subcutaneous Injection-Acrolein

The earliest study on acrolein carcinogenicity by Steiner et al. (1943) included several experimental groups. Acrolein (0.2 mg in 0.1 cc sesame oil) was injected subcutaneously once weekly for 24 weeks in 15 female "partly inbred albino mice" of the author's own stock. In addition, 15 female and 16 male mice from the same stock received 0.5 cc heated sesame oil three times at 4 week intervals. The "negative control" include 50 mg cholesterol in 0.5 cc (non-heated) sesame oil injected subcutaneously five times at 4-week intervals. The experiment was terminated at the end of two years. Three spindle-cell sarcoma cases were noted in the "heated sesame oil" groups while none were noted in the acrolein or "negative control" groups. The number of animals in this experiment was too small to draw any conclusion regarding the carcinogenic activity of acrolein in mice.

7.1.2 Skin Application-Acrolein

Salaman and Roe (1956) have tested 21 chemicals including acrolein for skin stumor initiating activity in S-strain mice. Fifteen mice (sex and age not specified) received weekly treatment via skin application of 0.5 percent acrolein in acetone over a 10 week period. Twenty-five days after the first acrolein application, the mice received a treatment of 0.17 percent croton oil. This was followed by 16 more weekly sin application of croton oil. The total acrolein dose was 12.6 mg per animal. Two of the 15 mice developed a total of 3 papillomas. Four of 19 mice receiving the same regimen of croton oil only developed 4 papillomas. The tumor incidence in the acrolein treated group is not a statistically significant response when compared with the croton-oil-only control. The small number of animals and the short duration of exposure are major shortcomings for these experiments thus making it impossible to draw a definite conclusion regarding the presence of absence of a carcinogenic response to acrolein treatment.

7.1.3 Inhalation-Acrolein

Eighteen 6-week-old Syrien golden hamster/sex/group were exposed via inhalation to 0 and 4 ppm (0 and 9.2 mg/m^3) acrolein 7 hours/day, 5 days/week for 52 weeks (Feron and Kruysse 1977). Six animals were killed at 52 weeks and 12 at 81 weeks in both treated and control groups. No increase in mortality and no statistically significant increase in tumor incidences were found in the treated animals compared to the control. The duration of the experiment was too short and the number of aimals too small to draw any conclusion from this experiment.

In separate experiments these authors tested Benzo(a)pyrene (BaP) and diethylnitrosamine via intratracheal instillation in addition to administration of acrolein to test the co-carcinogenic potential of this chemical. The results, however, fail to show an enhanced tumor response in those animals exposed to BaP (or DENA) and acrolein in comparison with the control which were exposed only to BaP (or DENA) and room air.

7.1.4 Skin Painting Glycidaldehyde (Metabolite of Acrolein)

Thirty 55-day old female Swiss albino mice received ither 2.5 mg glycidaldehyde, or 0.125 mg 7, 12 dimethyl-benz(a)anthracene (DMBA), as a positive control, in 0.25 ml acetone administered by skin painting. Three weeks later, 0.25 ml 0.1 percent croton oil in acetone was applied 5 days/week for the next 30 weeks. In addition to the one positive control of DMBA, there were three negative control groups (30 animals per group): 1) no treatment, 2) croton oil alone, 3) or acetone alone. Forty percent of mice treated with glycidaldehyde (12 mice) and 93 percent of those treated with DMBA (28 mice) developed keratoacanthoma after 30 weeks, whereas none were observed in any of the negative control groups. The time-to-tumor (first tumor) from the beginning of treatment was 5 weeks for DMBA, compared with 16 weeks for glycidaldehyde. Although keratocanthoma are benign, there is a potential for their progression to malignancy in some animals (Shamberger et al., 1974). This tumorigenic response is suggestive of a carcinogenic potential for glycidaldehyde in rodents.

7.1.5 Skin Painting and Subcutaneous Injection-Glycidaldehyde

Thirty 8-week old female ICR/Ha Swiss mice were painted with a 3 percent benzene solution of glycidaldehyde (100 mg solution/application) 3 times weekly for life. The median survival of these mice was 496 days. This compares reasonably well with the range of 324-583 days among this strain of mice under various other treatments. Sixty animals in a "negative-control" group receiving benzene as a vehicle had a median survival of 498 days. There were no tumors reported in the negative control group. Sixteen of 30 glycidaldehyde treated animals developed papilloma or carcinoma (8 with papilloma and 8 with carcinoma). The first papilloma case occurred 212 days after the initial glycidaldehyde treatment, and the first carcinoma after 338 days. There was both a statistically significant increase of skin tumor incidence and a shortening of latencies in this experiment.

Forty-one 8-week old female ICR/Ha Swiss mice were painted with a 10 percent acetone solution of glycidaldehyde (100mg) 3 times weekly for life (598 days). Six developed papilloma; squamous cell carcinoma subsequently developed in 3 of the 6. The median survival time of the treated rodents was 445 days. No skin tumors were seen in 300 control animals treated with acetone alone. The control animals had a median survival of 526 days (Van Duuren et al., 1967). There was a statistically significant increase in the tumor incidences with the glycidaldehyde treatment.

Three groups of 8-week old female ICR/Ha Swiss mice (110, 50 and 30 animals per group) were given subcutaneous injection of tricaprylin (vehicle control), 0.1 or 3.3 mg glycidaldehyde in 0.05 ml tricaprylin once weekly for life. Local sarcoma or squamous cell carcinoma occurred in 0/110, 3/50 and 7/30 of the control, low-dose and high-dose groups, respectively, (p for trend is less than 0.01 showing a "dose effect" response (Van Duuren et al., 1966). The median survival times for the control, low-dose and high-dose groups were 560, 593 and 472 days respectively. Among injection site tumors in the treated animals there were 2 fibrosarcomas and 1 squamous cell carcinoma in the low-dose group; and there were 3 fibrosarcoma, 1 squamous cell carcinoma, 1 undifferentiated sarcoma and 2 "papillary adenocarcinoma" in the high-dose group. There were significant increases in tumor incidence among those animals treated with glycidaldehyde.

Two groups of 6-week old female Sprague-Dawley rats received 1 or 33 mg glycidaldehyde in 0.1 ml tricaprylin solution subcutaneously weekly for life (50 and 20 animals each, respectively). Local sarcoma occurred in 1 of 50 low-dose and 2 of 20 high-dose animals. The median survival times were 558 and 539 days, respectively. In two control groups treated with tricaprylin 1 local sarcoma was seen (with the survival times ranging from 555 to 565 days). In 2 untreated controls (20 and 50 animals), no tumor was seen (Van Duuren et al., 1966, 1967).

7.2 SUMMARY

1. No epidemiological studies evaluating potential chronic health effects of acrolein are available

2. Animal studies on acrolein carcinogenicity are inadequate.
   a. Subcutaneous injection and skin painting studies were of inadequate design to draw any definite conclusions even though the authors' reported that there were no statistically significant response.
   b. An inhalation study in hamsters was negative with a 4 ppm exposure level although the design and conduct of the study were inadequate as a chronic bioassay for carcinogenicity. Thus, the results do not provide a basis for a definite statement about the human carcinogenic potential.
   c. There are no ingestion studies available.

3. Animal studies with glycidaldehyde, an acrolein metabolite, provide some evidence of carcinogenicity.
   a. Three skin painting studies in mice showed a statistically significant response of a benign tumor type which has the potential to progress to malignant tumors in animals.
   b. Subcutaneous injection studies in mice showed a statistically significant response for injection site tumors with a dose-effect relationship.
   c. Subcutaneous injection studies in rats showed a statistically significant local sarcoma response with a dose-effect relationship.

4. Several classes of chemicals, aldehydes and dienes, which are structurally or functionally related to acrolein and a metabolite of acrolein, glycidaldehyde are alkylating agents and show evidence of being animal carcinogens.

5. There are some positive results from mutagenicity studies in the bacterial systems, Drosophila larva and sister-chromatid-exchange studies of mammalian cells in culture (see mutagenicity section).

7.3 CONCLUSION

There are no epidemiological studies relating acrolein expsoure to carcinogenicity in the present data base. The skin painting and subcutaneous studies of acrolein are inadequate to access carcinogenic potential, similar studies of its metabolite, glycidaldehyde are, however, supportive of a carcinogenic potential. There are two different families of chemical compounds which may be functionally related to acrolein: aldehydes and dienes. The first group include chemicals such as formaldehyde and acetaldehyde, which are considered to be probable human carcinogens by EPA. The second group includes diene-vinyl compounds, such as ethylene oxide, acrylonitrile, vinyl chloride, and 1,3 butadiene. These have been classified as probable or known (vinyl chloride) human carcinogens.

Based upon the (1) structural relationship between acrolein and related compounds which are potentially carcinogenic to humans, (2) animal studies which suggest a carcinogenic potential for a known metabolite of acrolein and (3) the lack of epidemiological data, acrolein is considered to have "limited" animal evidence for carcinogenicity. The limited evidence designation derives from EPA's weight-of-evidence criteria in the EPA Guidelines for Carcinogen Risk Assessment dated August, 1986. With limited animal evidence, acrolein is classified as a Group C substance, meaning that acrolein should be considered a possible human carcinogen.

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8. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

The health effects of acrolein have been reviewed recently (Beauchamp et al., 1985; Izard and Libermann, 1978; U. S. Environmental Protection Agency, 1980). A U.S. Environmental Protection Agency Health and Environmental Effects Profile for Acrolein has been prepared recently in draft form. With respect to reproductive and developmental toxicity, the effects reported indicate that acrolein is embryo/fetotoxic, with reduced viability and growth retardation as major effects. Under certain conditions, teratogenic effects can also be produced. There is no evidence of direct effects on either the male or female reproductive systems. However, the evidence is not convincing that such effects cannot occur since no detailed examination has been done of the effects of acrolein on either the male or female reproductive system.

8.1 *IN VIVO* STUDIES

Epstein et al. (1972) reported the results of dominant lethal tests for 174 agents. Data for the individual agents were not presented except the levels of each agent that produced an increase in embryo loss. In that study, male mice received a single ip injection of acrolein. They were then caged with three untreated females each week for eight consecutive weeks. Females were sacrificed 13 days after being removed from the male. Observations were made on a number of pregnant females (mating not verified), total implants and early fetal deaths. Acrolein was reported to cause increased early fetal death and/or preimplantation loss at both levels tested (1.5 and 2.2 mg/kg). No other data were presented.

Bouley et al. (1976) exposed male and female (SPF OFA) rats continuously, by inhalation, to 0.55 ppm acrolein for 26 days. Mating was initiated on the fourth day of dosing and females were examined 22 days after initiation of cohabitation. No significant difference was detected in number pregnant, number of fetuses or mean weight of fetuses. No data were presented in that publication.

Claussen et al. (1980) tested the effect of acrolein in New Zealand white rabbits. Pregnant animals received iv injections of 3, 4.5 or 6 mg/kg on day 9 of gestation. On day 29, the fetuses were examined. At levels that cause maternal toxicity, a dose-dependent, statistically significant increase in percent resorptions was reported at 6 mg/kg. There were trends that were not statistically significant for the proportions of retarded and malformed fetuses to increase.

Claussen et al. (1980) also injected 10, 20, 40 microliters of 0.84 percent acrolein into the yolk sac of day 9 rabbit embryos in utero and examined the fetuses at day 29. Significant increases were produced in proportions of resorptions, retarded and malformed fetuses. Observed malformations included deformed and asymmetric vertebrae, spina bifida aperta, ribs deformed and accreted, and lack and fusion of sternum segments.

Using a different approach, Hales (1982, 1983) injected acrolein or diethylcyclophosphamide (metabolized to acrolein, but not phosphoramide mustard) into the amniotic fluid of day 13 rat embryos. Embryos were then allowed to develope in utero until day 20. Data presented for the diethylcyclophosphamide study indicate that approximately 50 percent of the control-injected embryos died. When acrolein was injected, 10 or 100 micrograms resulted in death of 100 percent and 98 percent of the fetuses, respectively. One microgram resulted in death of 65 percent of the fetuses and produced malformations in 86 percent of the live fetuses, while 0.1 microgram was no different from the control. Malformations produced by 1 microgram of acrolein included edema, hydrocephaly, open eyes, cleft palate, omphalocele, tail, and limb defects.

Injection of diethylcyclophosphamide into the amniotic fluid (Hales, 1983) was not lethal, but did cause malformations in 14 of 40 embryos. The types of malformations were similar to those produced by acrolein in embryos treated in the same way. In both of the studies by Hales (1982, 1983), it appears that the localized dose to the embryo is high compared to that from *in vitro* culture or in the *in vivo* studies in which dosing was done orally or by inhalation.

8.2 *IN VITRO* CULTURE STUDIES

Spielmann and Jacob-Muller (1981) removed embryos from mice at the 4-8 cell stage and cultured them in vitro with 100 nM to 1 mM acrolein to the blastocyst stage. Embryos that reached the blastocyst stage were cultured for another 120 h in medium that did not contain acrolein. No statistics are reported in this paper nor are the values for controls. Therefore, it is not possible to assess the sensitivity of the test system. Compared to controls, 1 μM acrolein appeared to reduce the percent of embryos producing an inner cell mass with two germ cell layers (79 percent). Ten μM acrolein inhibited attainment of inner cell mass (74 percent) and inner cell mass with two germ cell layers (34 percent). At 100 μM acrolein, no embryos reached the blastocyst stage.

Schmid et al. (1981) cultured more advanced rat embryos in vitro for 48 hr to assess the effect of acrolein in the medium on both embryo growth and differentiation. Embryos were explanted at day 10.5 of gestation. Acrolein levels of 100 and 150 μM produced a small, but statistically significant inhibition of growth. At 200 and 250 μM acrolein, growth and differentiation were severely inhibited, but no gross structural defects were observed. Culture in the presence of 350 μM cyclophosphamide, liver microsomes and NADPH (produces both acrolein and phosphamide mustard) produced embryotoxicity that included teratogenicity.

Mirkes et al. (1981, 1984) have conducted a series of experiments with rats that may explain the discrepancies between the previously-described studies. First, day 10 embryos were cultured in vitro for 24 hr with acrolein in the culture medium (Mirkes et al., 1981). Five μg of acrolein/ml reduced viability to 89 percent (not significant), while 10 μg/ml resulted in 100 percent embryo death. No malformations were seen. From those results, as with the results of Schmid et al. (1981), acrolein appeared to be fetolethal, but not teratogenic.

Subsequently, Mirkes et al. (1984) utilized dechlorocyclophosphamide (D-CP) and acrolein (separately) in the same culture system (Mirkes et al., 1981) and with ip injection of rats on day 10 of gestation. D-CP is metabolized in vivo to acrolein and dechlorophosphamide mustard (D-PM). D-PM did not produce any embryotoxic effects in the dose range used in these experiments. In vitro exposure of day 10 embryos to D-CP for 24-26 hr caused a reduction in growth measurements beginning at 12.5 μg/ml. At 25 μg/ml, the number of somites was decreased. A dose-dependent increase in incidence of malformations began at 6.25 μg/ml (33 percent) and reached 100 percent at 25 and 50 μg/ml. With acrolein (0.005-5.0 micrograms/ml), day 10 embryos were incubated in serum-free medium to investigate the possibility that acrolein was bound to macromolecules in the medium (or on membranes outside sensitive embryonic sites) and thereby prevented from reaching the sensitive sites. Under those conditions, a 2 hr pulse of 0.05 µg acrolein in the culture medium produced growth retardation. Decreased viability was also seen at all concentrations with complete lethality seen at 0.5 µg/ml and above. Abnormal embryos (abnormal flexion) were produced at all levels tested.

Mirkes et al. (1984) also injected D-CP (5, 10, 15, 20 and 50 mg/kg) ip into rats at day 11 of gestation. Fetuses were examined at day 20. The only effect was a reduction in mean fetal weight at 50 mg/kg. No mention was made about observations for maternal toxicity.

8.3 CONCLUSION ABOUT REPRODUCTIVE EFFECTS

With respect to reproductive and developmental toxicity acrolein is embryo/fetotoxic. There is no evidence of direct effects on either the male or female reproductive systems. However, the evidence is not convincing that such effects cannot occur since no detailed examination has been done of the effects of acrolein on either the male or female reproductive system. The embryo/fetotoxic effects have been observed in *in vitro* culture and embryo injection studies. The effects include reduced viability and growth retardation. Teratogenic effects can also be produced under specialized conditions. The important consideration appears to be that a sufficient amount of acrolein actually reaches the sensitive sites within the embryo or fetus. Possibly due to binding of acrolein to sulfhydryl and other reactive sites, as well as metabolism of the compound, fetal effects have not been demonstrated *in vivo* in the absence of maternal toxicity.

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